

How to make another omelette

Next weekend's economic summit at Venice cannot but be more important than its predecessors. Whether it will solve urgent problems is another matter.

GENTEEL convention has required, since the beginning of this decade, that the leaders of the seven most economically influential nations in the market-driven world should assemble regularly to brood on what might be made to happen next. There are two equally tenable, if cynical, opinions of the value of these occasions: they create the illusion that great issues are being tackled when they are merely being redefined (as when the 1979 petroleum price crisis led to an agreement to build strategic stocks of oil) and they give civil servants (but sometimes governments) an opportunity to add novel items to the international agenda (witness President Mitterrand's espousal of technology in 1981). This time it is improbable that negotiation and agreement, however reluctant, can be as lightly ducked.

None of the governments participating at the weekend can be indifferent to one issue that should not figure on the agenda of an economic summit at all — the question of what should be done at Geneva to fall in with Soviet suggestions that the time has come to get rid of nuclear weapons in Europe. The popular fancy, that the best kind of deal would be one in which missiles of intermediate range carrying nuclear warheads would be withdrawn altogether, and that there would also be a deal on rockets with even shorter range (although nuclear hand-grenades and their equivalents would still be allowed), is a simplifying approximation to the ideal that appeals to the Soviet Union (not at Venice), which the United States considers it must accept because it first put forward the suggestion, and which should persuade the British and French to come clean, and confess that their anxiety and interest is to plan for the time when the United States, out of self-preoccupation rather than indifference, washes its hands of Europe. When else will there be a chance to open that issue?

Urgency

There are also more urgent questions to be settled, the most immediate of which is that of whether the United States will allow Japan to emerge from the mercantile doghouse into which it has been compelled during the past few months by the US conviction that Japan is not playing fair on trade. As the world knows, the circumstances are curious, to say the least. The US administration is offended that it is in hock to Japan and (to a lesser extent) West Germany, but declines to acknowledge that it could not run a budget deficit of \$150,000 million, at least while its taxpayers are unwilling to bridge the gap by voluntary saving, without being in hock to somebody. After a great deal of shouting, Japan has now agreed to spend \$30,000 million or so on the stimulation of domestic demand, the immediate effect of which resolve would be to compromise the capacity of the Japanese to lend indirectly to the US Treasury. President Ronald Reagan has applauded this decision, apparently innocent of the degree to which Japanese intentions may be delayed by procedural matters and of the certainty that, if Japan were to be successful, US interest rates would have to rise still further to tempt other lenders to bridge the domestic US deficit. Who, at Venice, will have a slide-rule fitted out to tell what balance between US pride (protectionism) and profligacy (the deficit) will best suit most people's interests?

If the economic imbalance between the United States and Japan is in this sense an illusion (making it go away may bring economic growth to a halt) that between the United States (together with many other industrialized states) and the world's low-income countries is more substantial and more urgent. One sign of what may happen was the decision two weeks ago of Citibank, one of the strongest of the commercial banks in the United States, to debit its own capital account to the tune of \$2,500 million in a single quarter, to allow for losses on its loans to developing countries. One way or another, the other banks will be forced to follow suit. One consequence will be the partial impoverishment of the owners of the banks concerned. Another will be that low-income countries will come to seem even less credit-worthy than the alarms of the past few years have shown them to be. Whether or not the US commercial banks replace trade-surplus countries such as Japan and West Germany as the ultimate servicers of the US domestic deficit, the result will be that all low-income countries will be further impoverished. How can this weekend's economic summit solve that problem in the two working days at its disposal?

Trade

The simple answer is that it cannot. Quite apart from the lack of time, what is called political will is also lacking; the rich countries are so much preoccupied with their immediate interests that they do not have the courage to imagine where their interests might coincide with those of the low-income countries. The more arcane answer is that there will be an opportunity arising in the next few years to put the world's economic house in order — the planned three or four-year negotiation of new rules for the General Agreement on Tariffs and Trade (called GATT). The guiding principle is that nation-states signatory to the agreement should not discriminate among potential trading partners by favouring imports from one rather than from another. Although the spirit (and probably the letter) of GATT has frequently been broken by devices such as the Multi-Fibre Agreement by which rich countries have protected broken-backed domestic textile industries against cheap imports from elsewhere, there is no doubt that GATT has contributed to the most remarkable economic phenomenon of the past quarter of a century — the spectacular growth of international trade. The question that must be tackled in the next three or four years is whether the process will be extended to allow the low-income countries to earn a living for themselves.

There is a gentle irony in the origin of the forthcoming round of negotiations, which was originally inspired by the resentment of the United States at the shocking subsidy of European agriculture by the member governments of the European Communities; it was natural to ask that agriculture should be covered by the rules of free trade from which it is at present excluded. Cooler consideration has of course revealed that Europe and the United States protect their domestic agriculture by different mechanisms to roughly the same extent; the losers are none of them, but the low-income countries that might make a better living by growing food and selling it in a more open market. Meanwhile, the United States has found allies of its own in-



dustrialized kind in Western Europe, where there are many who wish to extend the free-trade rules to cover financial services, another of the issues that may yet appear on the new GATT agenda. What the industrialized countries have not yet understood is that they stand to make as much on the swings (of free trade in services) as they lose by the protection of their agriculture. If properly managed, the new GATT negotiations could ensure that there will be no losers, either now or in the future, but there would be conspicuous winners in the developing world. Low-income countries, to be represented for the first time at the GATT negotiations, will be keen to make that point.

Meanwhile, this weekend's summit meeting could marvelously prepare the ground. The past few months have seen too many assaults on the complacency of the well-to-do nations of the world for any of them to enjoy a self-confidence commensurate with its gross domestic product. What the world needs now is an acknowledgement by the richer creditor nations that their future prosperity requires that the low-income nations should be enabled to be richer than merely necessary to pay their debts. Curiously, it will help enormously if the seven heads of government can bring themselves to confess that this is the case.

Meanwhile, there is also AIDS (acquired immune deficiency syndrome), an international conundrum which is also, in its implications, economic. Throwing money at researchers will not, on this occasion, suffice. The need is to galvanize a search for an understanding of the distribution of the infection and of the mechanisms of its transmission as well as an appreciation by governments of the seriousness of the threat. Even if the issue is a little off the usual beaten track of governments at summit meetings, the time spent in drafting the appropriate paragraph (and making sure that it is understood by those who sign it) would be well worthwhile. □

Whose red face?

The European Communities' needlessly stringent radiation standards deserve ridicule.

ALL radiation is potentially harmful, right? (Everybody agrees.) And so the smaller the amounts of radiation that people ingest in their food, the safer they will be? (Right again.) So why not seize every possible opportunity to tighten the regulations that determine the amounts of radioactivity permitted in the food that people eat? This chain of argument is the most respectable rationalization of how the European Commission reached its decision last month (see *Nature* 327, 267; 1987) that the permitted limits for radiocaesium in foodstuffs should be fixed at 1,000 becquerels per kg for dairy products and 1,250 Bq per kg for other commodities. But this is only a thin excuse for having reached a thoroughly irrational decision whose immediate (and adverse) consequences, if any, will be economic, but whose long-term effect will be to bring into disrepute the careful process of setting radiation standards worked out over the past half century.

The origins of the Commission's proposals are political, not technical, and go back to the aftermath of the accident at Chernobyl in April last year. Several European governments then rushed to restrict the import from Eastern Europe of foodstuffs contaminated with fallout from the accident. West Germany plucked out of the air a working limit for radiocaesium of 600 Bq per kg, a quantity apparently determined more by reference to the quantities of radiation in Polish foodstuffs than waiting for clearance at its frontiers than by a deliberate consideration of the risks that would be entailed by allowing them to be put on sale. Just over a year ago, an ill-prepared meeting of a representative committee in Brussels found a compromise between the rigour of the West German standard and the earlier recommendations of committees which had calculated what would be the risks of ingesting radiocaesium, and which had concluded that working limits of between 9,000 and 30,000 Bq of caesium

radiation per kg of food would be appropriate: the European Commission eventually settled for a temporary limit on radiocaesium of 600 Bq per kg, with the understanding that a more permanent figure would be promulgated by the end of October this year.

That radiocaesium has been at the centre of attention is hardly surprising. Like iodine, caesium is among the more volatile of fission products; large quantities were indeed released from Chernobyl. But the principal fallout isotope of caesium, caesium-137, has a radioactive half-life measured in decades, forms chemical compounds which are soluble in water and, by an ion-exchange process, tends to hang about in the upper layers of soil. This is why radioactive caesium has reappeared in European grass in the spring a year after Chernobyl; it will remain, with a flush of reappearance every spring, for some years to come, if in declining quantities. The spring season, which is when grass grows most quickly, is that in which caesium will be most conspicuous. Those who drink milk or who eat meat derived from grazing animals will inevitably take in some caesium.

The good news is that caesium is not appreciably concentrated in particular organs of the body (as iodine is concentrated in the thyroid) and that its average residence time in the adult human body is estimated at 110 days (but is somewhat less for children). Despite the continuing numerical uncertainty about the chance that small doses of radiation from radionuclides systemically delivered will cause cancer, it is a much easier to calculate the relative risks of caesium-137 and other similarly distributed isotopes. The yardstick by which the standard of 30,000 Bq per kg of radiocaesium in foodstuffs is arrived at is the calculation that people perpetually eating food contaminated to this degree should incur a risk of contracting cancer which is statistically imperceptible compared with the natural risk of cancer.

So why should Europe now be anxious to settle for a more stringent limit? The Commission gives two reasons — the need to coordinate standards with those elsewhere (Scandinavia, for example) and the need to fix a limit that commands "public confidence". The first argument does not stand up. However stringent are the limits in force elsewhere, a stringent European standard will not by itself ensure that caesium is cleared from European soil or that European foodstuffs will comply with other people's standards. On the other hand, the new standard has the dubious advantage of justifying some of the potentially controversial practices of European governments in the present growing season; some British lambs from North Wales and Cumbria have been kept off the market (as were more last year), giving the authorities responsible the air of being vigilant (and the farmers concerned a sense of having a hole in their pockets). With a little luck, of course, these inconveniences will have blown over by next spring, when caesium concentrations in the soil will have further declined.

The Commission's argument about "public confidence" is potentially much more dangerous. The assumption seems to be that a show of stringency will give people who live in Europe a sense that their governments are zealous in their regard for public welfare. But what if, at some stage, there should be a nuclear accident in Europe. With the needlessly strict standard now proposed, much less damaging accidents than that at Chernobyl could make it necessary to take European foodstuffs off the market — or to relax the standards on the spur of the moment. Is that the best way to inspire confidence? It would be much more prudent of the Commission to promulgate a standard consistent with what the logic of the circumstances suggests, thereby helping a large and sophisticated population to appreciate that it must learn to live with radiation — and to thank its lucky stars that, for the time being, radioactive contamination of the food chain is well within the limits set for them. European governments, which will be required to approve the Commission's proposals during the summer, would be well advised to throw them out in favour of more rational proposals based on scientific recommendations. □

AIDS conference launched with controversial message

- Bush and Reagan speeches not well received
- Gallo puts case for HTLV-1 screening

Washington

THE Third International Conference on AIDS (acquired immune deficiency syndrome), running for five days in Washington DC from 1 June, was ushered in with a controversial call for widespread AIDS virus testing from President Ronald Reagan. In his first major speech on AIDS, delivered on the eve of the conference to the American Foundation for Aids Research, Reagan stopped short of demanding the compulsory testing of large sections of the population that he and conservative cabinet colleagues are thought to favour. Instead, he advocated compulsory testing for aliens and immigrants seeking permanent residence in the United States and inmates of federal prisons. But he called for "routine" testing — which could be refused without breaking the law — of couples seeking marriage licences, patients at clinics for drug abuse and sexually transmitted diseases and inmates of state and local prisons.

Reagan's announcement was met with some subdued booing from the audience, who had each paid \$250 to attend the fund-raising dinner, hosted by actress Elizabeth Taylor. The boos were a lot louder when Reagan's message was repeated the following day by US Vice-President George Bush at the opening of the international AIDS conference.

The 6,000 attending the conference had left little doubt where their support lay by

giving a standing ovation to US Surgeon-General C. Everett Koop, who spoke briefly before Bush. Koop has consistently supported the view of health workers, scientists and the Centers for Disease Control that mandatory, or even "routine" testing, is a waste of resources. Most AIDS cases have occurred among drug users and homosexuals who rarely marry and are unlikely to fall into the mandatory testing net. Information campaigns that persuade high-risk groups to come forward for testing are favoured by Koop, particularly as mandatory testing could inhibit drug users and homosexuals from seeking medical help.

Both Reagan and Bush went out of their way to praise Koop. Bush also emphasized the government's obligation to make the public aware of the risks of infection with HIV, the human immunodeficiency virus that causes AIDS. Pointing to the fact that several other countries had already mailed information on avoiding infection to every household, Bush said that the United States should learn from their experience.

By the time Bush had finished speaking, registration for the conference had reached the maximum permitted by fire regulations. The conference closed its doors amidst some chaos. By then there was one delegate for every eight cases of AIDS in the world (51,000 at the World Health Organization's latest count) and one member of the press for every 50 cases. Camera-men and photographers had been persuaded not to interrupt proceedings, which at least made events less of a circus than at the meeting's predecessor, in Paris last year.

Robert Gallo, on home territory, kicked off the scientific sessions with news of a novel AIDS-related virus. Isolated from a few Nigerians with AIDS or AIDS-like symptoms, the virus is only very distantly related to HIV or to the second class of AIDS-related virus, which is found largely in West Africa. Nor is the Nigerian virus related to HTLV-1, the retrovirus known to cause human leukaemia. Gallo predicted that other viruses were yet to be discovered. In particular, he predicted that a virus very closely related to HIV would be found in African monkey because, following recent evidence of the extent of sequence divergence between HIV and LAV-2, he no longer sustained the view that the former evolved from the latter in humans. Instead, HIV must have its direct

MRC allocates grants for AIDS research

London

BRITAIN'S Medical Research Council (MRC) is finalizing its first round of grant allocations from its £15 million, three-year programme of research into AIDS (acquired immune deficiency syndrome), announced in March (see *Nature* 326, 4; 1987). This year, MRC has £2.5 million available for its two programmes of research into drugs and vaccines. So far about a dozen laboratories have been selected, to receive a total of around £1 million, with the greater proportion going towards the programme to develop a vaccine. Applications from a further 12 laboratories are being considered.

Both programmes are being overseen by Sir James Gowans, secretary of MRC, who is also chairman of the vaccine committee. Professor Max Perutz, of MRC's molecular biology laboratory in Cambridge, is chairman of the committee on antiviral therapy. The two committees will meet monthly to identify new areas of work and to monitor progress. While stressing that the initiative was still at an early stage, Gowans said last week that the amount of interest being shown by the science community was "very encouraging". All the research so far commissioned has been in the public sector, although negotiations with two (unnamed) industrial laboratories are being undertaken.

With the vaccine programme, particular emphasis is to be placed on identifying systems in which candidate viral proteins can be expressed, and on the task of finding suitable animal models. MRC is not yet able to release details of specific projects that will receive support.

Gowans is satisfied that MRC has sufficient funds to ensure a viable domestic programme for AIDS research, but he would like to see Britain making "a more substantial effort" into studies of AIDS in Africa.

Simon Hadlington

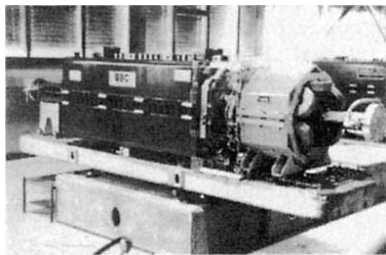
origins in a monkey virus.

Pointing to evidence that HTLV-1 can cause some degree of immunodeficiency and may well hasten the appearance of AIDS in patients doubly infected with that virus and HIV, Gallo suggested that it may be advisable to start extensive HTLV-1 screening. A considerable proportion of some populations of US drug addicts is infected with HTLV-1, he said.

Promise of the simple and reliable test that is needed, particularly in Africa, to discriminate between the two classes of AIDS-related viruses was held out by Erling Norrby of the Karolinska Institute. By the time the great AIDS show has shifted to Stockholm next year, the reality of that prospect should be clear.

Peter Newmark & Alun Anderson

LEP magnet in place



LEP — the large electron-positron collider project at CERN — proceeds apace. Today (4 June), the first magnet is being placed in the subterranean tunnel of LEP in a ceremony attended by Jacques Chirac, the Prime Minister of France, and Pierre Aubert, the President of Switzerland. The magnet shown is one of a thousand that will guide the electron and positron beams on their 27-km route under the Jura mountains in France. LEP, due to operate for experiments from Spring 1989, promises exciting results in high-energy physics. □

Japanese poised to dominate in superconductors as well?

Tokyo

WHAT is happening in high-temperature superconductor research in Japan? Is Japan Incorporated ready to conquer the world? Not yet. Research is under way on a broad front but is largely uncoordinated, as government agencies and ministries manoeuvre to stake out territory and preserve their interests.

Since a group of researchers at Tokyo University confirmed superconductivity at about 30 K in a copper-oxide ceramic at the end of last year, research on the new materials in Japan has exploded. The April and May special issues of the *Japanese Journal of Applied Physics* contain nearly 200 papers on the new superconductors by several hundred scientists in about 80 laboratories throughout Japan. And barely a day goes by without the announcement of another 'breakthrough'.

Much of the effort, however, involves unnecessary duplication. On 4 March, the Science and Technology Agency's National Research Institute for Metals in Tsukuba announced that it had succeeded in making a high T_c ceramic, only to find that the same announcement had been made the day before by its Tokyo research institute. Researchers at the two laboratories were apparently unaware that they were making the same ceramics even though both had representatives on the agency's research committee on high-temperature superconductors. The committee, with representatives drawn from the universities, industry and the agency's laboratories, was formally set up in February but began informal meetings at the end of last year. The committee's principal job is to organize workshops and symposia; the first symposium was held on 1 May with I. Bednorz of IBM Zurich as guest speaker.

According to Koichi Kitazawa of Tokyo University, one of the committee members, the Science and Technology Agency has been very quick off the mark and has played an important role in disseminating news. The right 'atmosphere' to win financial support from the Ministry of Finance is being created. The new superconductors also provide a golden opportunity to boost the agency's National Research Institute for Metals whose fortunes have been declining along with those of the iron, steel and aluminium industries. On 28 May, the agency announced the establishment of a 50-man research centre at the Tsukuba site next spring to carry out basic and applied research on the new ceramics.

When it comes to funding research, though, the agency has been beaten off the mark by, surprisingly, the Ministry of

Education. Casting aside its conservative image, the ministry has taken the extraordinary step of extending a major fixed-term grant, something that has only previously been done in the case of a natural disaster. A special research project to investigate new superconducting materials headed by Professor Nakajima of Tokai University (formerly of Tokyo University) is to be extended at a cost of ¥180 million (\$1.3 million). In addition, Professor Kazuo Fueki of the Tokyo University group has been awarded ¥36 million by the ministry for this fiscal year (April 1987–March 1988). And Professor Shoji Tanaka, leader of the Tokyo University group, is a strong candidate for one of the ministry's special distinguished grants which are announced at the end of this month. Running from three to five years, the grants are usually worth ¥100–200 million (about \$1 million).

But the biggest government backer of technology development, the Ministry of International Trade and Industry (MITI), has yet to show its cards. MITI has long been a strong supporter of superconductor research. Companies such as Toshiba and Hitachi, nurtured in MITI's magnetohydrodynamic project, and aided by Japan National Railway's bid to build a high-speed train levitated on superconducting magnets, have gone on to supply many of the magnets for US particle accelerators. Josephson junction research has also been supported within the national supercomputer project. Although US companies, including IBM, abandoned similar work, researchers at NEC, Hitachi and Fujitsu remain confident that a Josephson junction computer can be built by the twenty-first century.

MITI money is also being funnelled to private companies through the Electric Power Central Laboratory in Tokyo, thereby allowing eight companies all to continue production of conventional superconducting wire, despite the comparatively small size of the Japanese market. It was from them that the first reports of the manufacture of high-temperature superconducting wires emanated. First off the mark was Fujikura Densen with La-Sr-Cu oxide encased in drawn-out tubes of copper and steel. Then on 2 April, Toshiba released pictures of wire fashioned from Y-Ba-Cu oxide. Although the current-carrying capacity of the wire was initially very low (a few amps per square centimetre), within a month Toshiba had reached 520 A cm⁻² and now Hitachi claims the record at 4,000 A cm⁻² for ceramic wire (0.8 mm diameter) encased in silver.

Despite these projects, however, MITI

Just five years from superconductor cable

Washington

THE US Department of Energy (DoE) is showing unusual alacrity in pushing for research on the new superconductors. Motivated by constant but uncertain rumours of furious Japanese activity, DoE officials have taken some considerable steps towards an organized national effort in this new technology.

A series of conferences to promote collaboration between the national laboratories, universities and industry is already under way, but DoE has now gone beyond this cheerleading role and has given Argonne National Laboratory a specific brief to produce a practical superconducting wire, operating in liquid nitrogen, in five years. (Argonne researchers have already made 'wires' by embedding superconducting grains in a plastic base.) The aim of the programme, in which Brookhaven and Ames (Iowa) Laboratories will also participate, is to make a cable suitable for electric transmission lines.

DoE also announced last week that it is setting up a computerized database to help US scientists to cope with the huge flow of results. Secretary of Energy John S. Herrington said that the normal channels of scientific communication are being overwhelmed, and that the DoE, by expanding its existing systems, could expedite the flow of information. The database will be accessible through electronic mail, and will be open to anyone who pays an entry fee.

The impetus for these initiatives is apparently coming from the top. At DoE headquarters, 'research applications' and 'Japan' are whispered in the same breath, and the current political climate is ideal for any venture which seeks to encourage US industry.

David Lindley

has yet to formulate a policy on the new superconductors. Some of the problems are internal. There are fears that a new project may siphon off funds from projects already established — to the Ministry of Finance, superconductors are all the same be they ceramic or otherwise.

After a hearing of the science and technology committee in the lower house of the Diet on 28 May, Professor Shoji Tanaka called for a national project to develop applications, such as a magnetically levitated train. Also present at the hearing was Yoshihiro Kyotani, head of Japan's linear motor car (MAGLEV) project. But ruling party politicians suggested that the project should be international and might even be proposed at the Venice summit next week.

Tanaka denies that he is suggesting that Japan set up a research association similar to the well-known VLSI (very large-scale integrated circuit) project of the late 1970s

Superconductivity at room temperature

New Delhi

SCIENTISTS at the National Physical Laboratory (NPL) in New Delhi are proudly claiming to have found the hottest ever superconducting oxide phase. It shows superconductivity all the way up to +26 °C (299 K), or room temperature.

The development was officially made public before publication in a scientific journal in an attempt to ensure its legitimate place in the race in superconductivity both in India and elsewhere. Indian scientists frequently complain that although they are keeping pace with the latest developments in Japan and the United States, their work is often dated by the time it is printed in international scientific journals.

The NPL discovery was made in multi-phase samples of $Y(Ba,Sr)_2Cu_3O_x$ prepared by the direct oxide-mixing technique. The typical resistance-versus-temperature curves show a sharp drop in resistance above 230 K followed by a gradual metal-like decrease of resistance with temperature. A study of the inverse a.c. Josephson effect revealed the presence of a phase superconducting up to +26 °C. "It is the hottest superconducting phase observed so far", said Dr A.V. Narlikar, the leader of the NPL team.

Because the sample had many phases, the studies were repeated in several different samples. In each of them, superconductivity was found to persist up to temperatures of 15 to 26 °C, said Narlikar. Studies also showed that the 26 °C phase constituted the bulk of the sample. Narlikar said his team is now working on isolating this phase. "When we do that, we will really have a room-temperature superconductor", he said. K.S. Jayaraman

which drew together Japan's electronics giants with MITI support and helped power the conquest of world semiconductor memory markets. But it is no secret that Tanaka has close connections with Japanese industry, in particular Toshiba and Hitachi where several of his present and former students carry out research.

Other scientists who are on the government/industry university committee established by MITI to study the new superconductors doubt that the ministry will form a research association. Rather, they think that a medium-scale project under the category "basic research for future industries" may be possible. These projects which cover new materials, biotechnology and new molecular devices (including biochips) typically receive funding of a few thousand million yen (around \$10 million) per year. But the earliest such a project could be set up would be 1988, and consensus would have to be reached

in MITI within the next few months.

Meanwhile, research in industry is largely "free-style" with no particular coordination, government or otherwise, according to Dr Janshen Tsai, supervisor of the advanced device research laboratory at NEC. NEC has fewer than 10 researchers working full-time on the new superconductors but there are about 30 or 40 part-timers and many more are interested in joining the research, which covers primitive Josephson junction devices and thin films.

Researchers at Toshiba's Research and Development Centre in Kawasaki, on the other hand, seem to be interested primarily in wires and thin films, and they have no intention of pursuing Josephson junction research. Osamu Horigami, Toshiba's chief research scientist at the centre's energy science and technology laboratory, has 28 researchers working on superconductors and cryogenics and they are collaborating with scientists in the metals and ceramics laboratory of the same centre. Horigami says his laboratory began investigating superconducting ceramics about six years ago in collaboration with Professor Tanaka of Tokyo University — but they gave up when they reached a critical temperature of only 18 K.

How much money are these companies putting into the research effort? Company officials will quote only the percentage of total sales devoted to all research and development (8–7 per cent for Toshiba and 10 per cent for NEC). But Dr Ushio Kawabe of Hitachi says that in general they budget about ¥10 million (\$70,000) per researcher per year.

Patents are being sought apace. Sumitomo Electric Industries, a large wire and cable manufacturer, is reported to have applied for 800 patents on superconducting technology covering materials, processing and application. Many of the leading researchers in Japan have also taken out patents, although nobody knows who was first.

Tanaka fears that Japan may once again be criticized for failing to contribute to basic research. He and his colleagues have been publishing heavily to make the world aware of their efforts as students grind out ceramics in the laboratory (now up to processing 48 samples a day). And to drive home the point, 1,000 copies of the April special issue of the *Japanese Journal of Applied Physics*, weighing 0.7 tonnes, were airfreighted to the United States and distributed free of charge at the Material Research Society meeting in San Francisco. Mitsui Co Ltd agreed to cover the ¥3 million (\$20,000) air freight costs as a "contribution to basic science".

Other Japanese researchers are less concerned about such matters. "Our interest is how to get wire and devices using this material", says Horigami of Toshiba. David Swinbanks

Supercomputer stand

Bangalore

US efforts to limit high technology exports on the grounds of national security may jeopardize the prospects for an Indo-US supercomputer deal. India wants the United States to supply a Cray-XMP-24 model, but the Reagan administration's security adviser, Frank C. Carlucci, is urging it to accept the Cray-XMP-14. In explanation, Robert Dean, the National Security Council's staff specialist on advanced technology transfer, says "We know this supercomputer [Cray-XMP-24] is something that the Soviets would very much like to get".

Carlucci says India and the United States have agreed on safeguards for the supercomputer as well as increasing levels of US cooperation in economic and technological fields. In New Delhi, Rajiv Gandhi's government, rocked as it is by political controversies, has yet to find time to assess the US offer. R.R.

FBA labs are reprieved

London

STAFF at the Freshwater Biological Association (FBA), threatened earlier this year with up to 30 redundancies because of a shortfall in income of around £400,000, have won a temporary reprieve. Following discussions between FBA senior management and the Natural Environment Research Council, which provides the bulk of FBA's funds through an annual grant-in-aid, 12 staff will now lose their jobs. The situation will be reviewed at the end of August. FBA's future now depends on its ability to increase its income from private contracts. The association, whose 103 staff study the ecology of rivers and lakes at laboratories in Dorset and Cumbria, fears that too much short-term commissioned work will result in a gradual abandonment of longer-term, less immediately lucrative studies. S.L.H.

Erasmus is adopted

London

TWENTY-FIVE thousand students could benefit from a new European Community Action Scheme for the Mobility of University Students (ERASMUS) over the next three years. The scheme has a budget of £59 million. The new grants will be available to European Community students wishing to study in other member states. Despite its name, the scheme will be open to students in all forms of higher education and there are to be no subject restrictions.

A European University Network will be set up to promote inter-university student exchange programmes and an improved system of academic comparison will assist in the placement of foreign students. A pilot scheme to be run in twenty universities will try out a 'credit transfer' scheme like that used in the United States. S.J.H.

Students mar anniversary

Munich

THREATS of violent protests at the West German University of Göttingen have forced officials to cancel ceremonies planned for 26 May to celebrate the university's 250th anniversary. A student "strike council" had vowed to blockade the doors to the university in protest against massive budget cuts proposed by the government of Lower Saxony.

A financial crisis has forced across-the-board cuts in Lower Saxony's budget. The Education Ministry has been asked to shoulder some of the burden by reducing expenditures by a proposed DM 130 million over the next four years. The cuts are to be achieved by abolishing 371 tenured positions out of a total of 10,900 and reducing the budget for shorter-term positions by 3.5 per cent. Furthermore, students who study for more than a specified number of semesters are to pay fees of DM 500 per semester of further study. At present, students pay no fees, however long they study.

It seems to have been this measure that loosed the wave of protest in Göttingen. The university administration agreed in principle with the protesters said speaker Gerhard Gizler. University President Norbert Kamp wanted to go ahead with the ceremonies, but was ordered to cancel them by the government of Lower Saxony.

Lower Saxony speaker Joachim Werren justified the introduction of student fees as a way of reducing the number of people who "take advantage of their student status" to obtain reduced insurance rates and other benefits. This claim was rejected by the *Westdeutschen Rektorenkonferenz* (WRK), the organization of university heads. A spokesman for WRK explained that most students have to work to support themselves and it is for that reason that they stay in the university so long.

The proposal to cut back on personnel has encountered an equally critical response from the Public Servants' Union, which has denounced them as cuts by the "lawnmower method" rather than a response to programmed goals. But an acceptable compromise might be found, said Gizler, if the universities themselves are allowed to choose where the cuts are to be made. It might be wiser, he said, to offer extra support to a renowned university such as Göttingen — at the expense of other institutions — rather than reduce it to mediocrity.

Further student protests might be touched off if Lower Saxony's cabinet does not modify its original proposal. A decision is expected on 2 June.

Steven Dickman

Ambitious plans for CNR give hope to Italian scientists

Rome

ITALIAN research is to be fertilized by the injection of a billion lire (£500 million) over the next five years. On 28 May, the Comitato Interministeriale per la Programmazione Economica (CIPE), the government body for economic planning, approved investment in ten projects devised by the Consiglio Nazionale delle Ricerche (CNR), the Italian research council.

In the ten CNR projects, apart from new discoveries in fundamental science, it is expected that there will be new products to market, especially in biotechnology and fine chemistry. Materials research is expected to yield advanced high-performance products, and telecommunications various experimental prototypes, while a supercomputer programme is designed to help integrate CNR projects with the European Esprit programme and to enable Italy to keep up with the latest trends in artificial intelligence. Other areas included in the plan are robotics, electro-optics, fine chemistry superconductors, cryogenics, building materials and town planning.

The projects will span the period 1987–91. They will involve 1,250 research groups at CNR institutes, universities and

industrial laboratories and will occupy 23,400 man-years of effort.

Senator Luigi Granelli, the minister of research, says that state expenditure will be increased by a contribution of 48 per cent from industry, which means that a total of a million million lire will be devoted to research and development. One objective of the new plan is that there should be a public incentive in specific fields for a deeper involvement of private companies in advanced applied research. Granelli has been minister of research since 1983, and the scientific community hopes he will be reconfirmed in that position after the elections on 14 June.

The approval by CIPE will enable CNR to make 600 grants to researchers in the fields now specified; there will be a further 500 contracts from collaborating industries, according to Professor Luigi Rossi Bernardi, the CNR president.

During the same CIPE session, the Italian government approved 76,000 million lire (\$50 million) for the 1.5 GeV synchrotron radiation source to be built at Trieste (see *Nature* 327, 5; 1987), while a first share of 209,000 million lire has been allocated for this year's start on the national research programme in biotechnology.

Paola De Paoli

Time Inc. runs out on *Discover* magazine

Washington

DISCOVER, the last survivor of a boom in popular science magazines, has been sold, giving its publisher, the massive Time Inc., its first magazine failure in recent memory. Declining interest in science seems not to blame, for *Discover's* circulation is close to 900,000. Rather, it seems that advertisers are not much interested in the kind of people that read science magazines. *Discover* could not continue at Time Inc. without a big rise in advertising revenues.

The new owner, Family Media Inc., publisher of such magazines as *Health*, *Savvy* and *Homeowner*, will pay \$26 million for *Discover*. The first issue under its control will appear in September, but both that and the following issue will rely on material already in preparation by *Discover's* staff. After that, the magazine may change, but Family Media Inc. has yet to announce an editorial policy or appoint an editor-in-chief.

The prospects for *Discover* at Time Inc. had seemed to improve last year after \$5 million was spent to buy up two competitors, *Science 86* and *Science Digest*, and offer their readers subscription transfers. But even that boost proved insufficient —

within Time Inc.'s structure, at least. Time Inc. is known for the lavish scale of its operations. *Discover*, a monthly, had a staff of 74, including 37 on editorial. *Discover's* stablemates, *Time*, *Life* and *Money* (magazines), could afford to do things in style. *Time's* advertising revenue alone last year was \$430 million.

Discover could manage an advertising revenue of only \$7 million last year. Prodigious efforts had, according to *Discover*, generated a 50 per cent rise in the first half of this year, and brought in a handful of cigarette and liquor advertisements. But big advertisers continued to regard *Discover* readers as unlikely to buy their products. Home computer advertisers had mainly fled to the specialist computer hobby magazines.

Under Family Media Inc.'s ownership, revenue will be sought by offering advertisements in tandem with other magazines in the group. The editorial staff will be lean. Some of *Discover's* current staff will be kept on and a few, particularly those on the management side, remain at Time Inc. But most will be taking severance payments, described by staff as generous, and looking for work elsewhere.

Alun Anderson

Radice labels the city technology colleges "a miserable flop"

London

THE British government — still on its way to a comfortable election win according to the opinion polls — is trying to boost its innovative experiment on industry-funded secondary schools, in the face of continued opposition from the Labour Party and local authorities. A special charitable trust has been set up to push the establishment of what the government is calling "city technology colleges", paid for by industry and geared towards science and technology.

The new colleges would be owned and run by industry, outside local authority control, and each would cater for up to a thousand 11- to 18-year-olds in inner city areas. Sponsoring companies would use their own staff as teachers, and would develop a curriculum to meet their own needs. According to Mr Kenneth Baker, Secretary of State for Education, at least 25 per cent of teaching time would be devoted to science and technology.

Baker hopes to set up 20 city technology colleges by the end of the decade, if he can get industry to respond. So far, the government has approached nearly 4,000 companies, and three have agreed to sponsor new secondary schools to the tune of £1 million: the Anglo-US conglomerate Hanson Trust at Solihull in the West Midlands; Dixons Group, operator of a national chain of retail electrical goods stores, in South Yorkshire; and Sir Philip Harris, chairman of the Harris/Queensway furniture group, at Wandsworth in London.

Of the three, only the Solihull proposal has a definite site. In other areas, the education authorities have not been keen to turn over their property. But the government has now indicated that it will use legislation to force local authorities to make land or buildings available.

A search for sites and for more sponsors is being led by the City Technology Colleges Trust, a group of educationalists and industrialists. But the Labour Party is pledged to refuse to support the new schools if it becomes the government after the 11 June election. Labour's education spokesman, Giles Radice, calls industry's response "abysmal", and adds, "the city technology colleges look set to be a miserable flop".

Labour does not like the idea of a lack of local authority control over the technology colleges, and it is also against the selective admission policies that would be applied. The Labour-controlled Association of Metropolitan Authorities has also come out in opposition.

Teachers' unions are resisting the new institutions. The recent conference of the

National Union of Teachers voted to organize a consumer boycott of sponsoring companies such as Dixons. The teachers fear that industry-funded colleges would cream off science and mathematics teachers and motivated students from state schools.

But some businesses are sceptical, and do not see why industry should fund schools at such a high level. The reward for companies, according to the government's glossy promotional brochure, will be "richer opportunities for good education in the cities and an enhanced contribution to the vigour and prospects of the communities there". However, three of Britain's largest companies, IBM, ICI, and BP, still remain unconvinced and have declined to provide backing for the new colleges.

The idea of better science education is laudable, says Professor Denis Noble of the Save British Science organization. "But an improvement is needed for at least 1,000 schools, not just 20". The danger, he adds, "is that this programme may make us complacent and divert attention from the larger problem. It's a typical English solution to massive education problems: improve some schools at the top of the system and forget about the rest."

Kathy Johnston

Britain seeks to share telescope costs

London

BRITISH astronomers are seeking to share the £350,000 annual cost of their UK Schmidt telescope with Australia. The proposal, which would halve the viewing time for Britons, is aimed at "value for money", says Dr John Litt of the Science and Engineering Research Council.

"International collaboration can help us by sharing the costs and bringing in another country's resources and expertise", Litt says. The council last month sold a share in its new James Clerk Maxwell telescope in Hawaii to Canadian astronomers as part of its economy drive.

The UK Schmidt telescope has been operating at Siding Spring Mountain in South Australia since it was built by the Royal Observatory Edinburgh in 1973. A wide-angle optics telescope, it is often used in conjunction with the nearby Anglo-Australian Telescope which operates under an intergovernmental agreement. The equipment is used in quasar discovery work, and its observations identified the progenitor star in the spectacular supernova explosion earlier this year.

Both the British and the Australian governments would have to agree on the cost-sharing move, but with each country facing an election there could be some delays. "This is hardly a high priority election issue", Litt notes.

Kathy Johnston

Somebody missing from Nobel line up?



FROM the left, Max Perutz, John Kendrew, Aaron Klug, James Watson, César Milstein, Fred Sanger, Hugh Huxley and Sydney Brenner pose at the 40th anniversary of the UK Medical Research Council's first foray into molecular biology. All carried out pioneering work in the council's Unit for Research on the Molecular Structure of Biological Systems, founded in 1947 at the Cavendish Laboratory in Cambridge, or in its successor, the Laboratory for Molecular Biology which was opened in 1962. Absent from the group is Francis Crick. It was Watson and Crick who, in 1953, became the first members of the group to win Nobel Prizes. They were followed by Perutz and Kendrew in 1962, Sanger in 1980 (adding to his 1958 Nobel prize for work carried out before he joined the Medical Research Council), Milstein (and Georges Köhler) in 1984 and Klug in 1985. The laboratory remains a centre of excellence for molecular biology but the days when it was unrivalled are long since past. History cannot be repeated, but the question for Aaron Klug, who took over from Sydney Brenner as director of laboratory last year, is how to maintain even its current standing in the face of the relative decline of UK science. □

News media making the most of Earth observation satellites

Washington & Paris

WHEN the French launched the Earth-observation satellite SPOT, in 1986, they thought their customers would be farmers, geologists and land-use planners. Their expectations were met, but they also found demand for SPOT images from an unexpected quarter: the media. Images from SPOT (and from the US Landsat satellites launched earlier) have opened up a whole new set of possibilities for the media, including the chance to look at pictures of Soviet nuclear and radar facilities and have a try at "do-it-yourself arms treaty verification", as a Spot Image representative put it. Demand has been such that there have been calls for the launch of a dedicated "media satellite". This possibility, and the potential conflict between the free availability of images from space and "national security and foreign policy interests" are the subjects of a new report from the US Congress's Office of Technology Assessment.

Over the past two years, US and European media have shown the public SPOT and Landsat images of the Chernobyl nuclear plant, a Soviet naval base on the Kola peninsula, Libyan missile sites, a nuclear processing facility in Pakistan, the Soviet nuclear test facility at Semipalatinsk, the battlefields at the Iran-Iraq border, several Soviet space and airforce bases and the Krasnoyarsk radar in the Soviet Union, which some think violates the terms of the Anti-Ballistic Missile Treaty. Needless to say, none of these places welcomes journalists.

SPOT (Système Probatoire d'Observation de la Terre) has provided the most detailed images. Its resolution is 10 m in black and white and multi-spectral sensors can help pick out objects that differ in any colour band. Landsats 4 and 5 are capable of 30-m resolution.

Spot Image's policy has always been one of distribution without discrimination: "We sell to anyone, whoever they are, without restriction" according to a spokeswoman at their Toulouse headquarters. Eosat, the private company formed by RCA and Hughes Aircraft to take over the Landsat satellites developed by the US National Aeronautics and Space Administration (NASA) has a similarly liberal view.

From time to time, most recently in a report from the US Commerce Department, the Reagan administration has shown signs of discontent about the uncontrolled flow of information from Earth satellites. Images from SPOT and Landsat are not actually of strategic importance. Both the United States and the Soviet Union have far more powerful spy satel-

lites in space. But the media might learn things governments do not announce. Buildup of supplies and troops for military operations (such as the 1983 US invasion of Grenada, for example) could be spotted from space and deprive an operation of the critical element of surprise. Information considered sensitive by foreign governments might also be revealed — construction work at nuclear power plants indicative of weapons development, for example — and make it more difficult for diplomacy to resolve problem. Data might even be misinterpreted by the media and help precipitate a crisis. SPOT images of the nuclear testing facility at Semipalatinsk are said to have been misinterpreted by US television networks as showing that the Soviet Union was about to recommence nuclear testing.

Bigger mistakes, at times of tension, could help trigger a conflict, according to the report. But satellite data also provide a means of checking public statements. The US administration's claims that the Soviet Union has violated nuclear arms treaties has been disputed from satellite image analysis as well as from seismic data.

SPOT and Landsat have disadvantages for news gathering. Neither can provide coverage in real time. Images of Chernobyl were obtained 24 hours after the accident there, but only because there hap-



Within 24h the Western media had this Landsat image of the stricken Chernobyl plant (centre of the rectangular-shaped area).

pened to be a satellite in position and because other activities were suspended. Rapid news gathering would need a minimum of two 'Mediasat' satellites and either a chain of ground stations or data-relay satellites in space, according to the report. Resolution would also probably have to be improved, to a minimum of 5 m. But a Mediasat would be very expensive and is unlikely to be built in the near future, according to the report. That leaves some time to work out legal problems of a public eye in the sky.

Alun Anderson & Peter Coles

Commercial Newsgathering from Space, OTATM-ISC-40, is published by the Office of Technology Assessment, Congress of the United States, 27 May 1987.

Mutual assurance for space launches

Paris

WITH the failure of two of the last four Ariane launches, failures in the US Delta and Titan rockets and the explosion of the Challenger space shuttle, all during a 12-month period, the space insurance market is licking its wounds. Having already settled record claims during the previous 12 months, the market may never fully recover. Satellite corporations are now thinking of ways to beat insurance companies, weighed down with losses, at their own game.

Space-insurance brokers have had to fix a ceiling of about \$100 million on the total risks they are willing to underwrite, while asking a hefty premium, often as high as 25 per cent. Although Arianespace, responsible for the production and marketing of Europe's Ariane rocket launchers, has formed its own insurance subsidiary (S3R) with more attractive rates for its customers, some companies may boycott the insurance market altogether. The most radical option, not to insure the satellite at all, has already been taken for some of the previous Ariane launches.

A more prudent alternative, now under discussion between the bigger, international corporations, such as Eutelsat, Inmarsat and Intelsat, is to create a mutual insurance society, where each member puts a given sum into a kitty for every launch any member has booked. If the launch fails, the costs are shared between members up to a previously agreed limit. If, on the other hand, the launch succeeds, the 'premium' is held over for the next launch and so on. Given the current risk, for example, such a system would enable members to absorb one failure in four at a far lower cost than if a separate premium were paid to an outside company each time.

In the long term, all depends on the failure rate of the next few launches, not only of Ariane but also of Soviet and Chinese competitors. It is almost inconceivable that losses will continue to occur at the rate of the past three years. A series of successes would bring premiums down again, making these radical alternatives to conventional insurance less attractive.

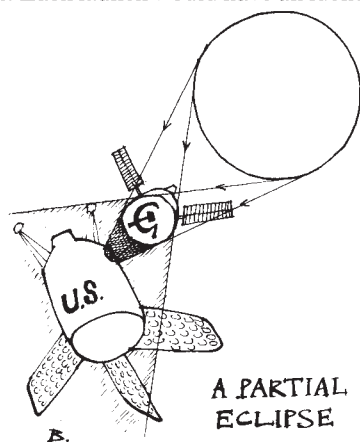
Peter Coles

Plans for Mars impress

Washington

THE embattled US space programme seems to be losing ground yet again, judging by a recent announcement of Soviet intentions. At the International Conference on Solar System exploration, Pasadena, California, senior Soviet scientists described ambitious (but so far unapproved) plans to launch a comprehensive exploration of Mars during the 1990s.

Four missions are on the drawing board: a balloon explorer, a rover, a lander and sampler, and a retrieval mission. Each launch would have an identical



backup, making necessary a total of eight launches on the large Proton rocket. These proposals are still under detailed review, and have yet to be formally presented to the Soviet government, but Soviet scientists are apparently confident that at least some parts of the programme will leave the ground. The balloon mission, which may include a small rover, could be sent off as early as 1992.

US scientists at the meeting were clearly impressed, not only by what the Soviets revealed of their long-term thinking but also by the fact that they revealed it at all.

The contrast in the level of optimism of US and Soviet space scientists showed up at a meeting of the National Aeronautics and Space Administration (NASA) in Washington last week, where a large committee met to find ways of revitalizing NASA's overall goals. The key words were 'augmentation' and 'leverage' which, in combination, seem to mean trying to add to existing plans in inexpensive but visibly productive ways. The committee expressed its pleasure that the collaboration agreement signed in Moscow earlier this year enabled NASA to gain control over the entrepreneurial spirit behind recent US-Soviet projects. Any US collaboration with the Soviets on the latest Mars ventures would have to be negotiated through high-level meetings.

David Lindley

California introduces methanol for cars in pollution fight

San Francisco

IF California were a nation, it would be the world's third largest consumer of gasoline (motor fuel), after the United States and the Soviet Union. No wonder, then, that it has been a leader in fighting air pollution and, with 22 million automobiles, has enacted tougher auto-emission regulations than the US Federal Environmental Protection Agency. The latest plan, which may set trends for the rest of the world, is to introduce methanol as an alternative to gasoline.

Under a scheme announced last week, the California Energy Commission and ARCO Corporation, the state's largest gasoline retailer, agreed to begin selling methanol at 25 southern California stations by the end of next year. A similar commitment for northern California is expected from Chevron USA Inc. The state will help things along by buying 5,000 government cars that can run on either gasoline or methanol, according to the California Energy Commission. If Californians follow this lead, the commission hopes 10-15 per cent of vehicles within the state will use methanol within a decade.

Methanol's biggest advantage is that it produces half the oxidants and particulates of gasoline. And it is easy to make

from coal and natural gas which the United States has in abundance. Most US automobile manufacturers are developing flexible-fuel cars that can use either methanol or gasoline in the same tank, and they are not expected to cost much more than conventional cars. The only problem is that methanol produces three times as much formaldehyde, a potential carcinogen, as gasoline. But it is not technically difficult to design a converter that turns formaldehyde emission to water vapour and carbon dioxide.

Marcia Barinaga

• Britain, France and Italy are close to agreement on limits for car exhaust emissions. The European Economic Community (EEC) proposed complex regulations two years ago to limit emissions of hydrocarbons, sulphur and nitrogen oxides. Member states have been arguing since then, with motor-manufacturing nations seeking compromises on standards and timing; meanwhile, West Germany and the Netherlands have already implemented the regulations. Under the EEC timetable, all small and medium-sized cars would have to conform to the new standards in stages by October 1991. The UK government is still resisting a proposal on large cars which would involve catalytic converters.

Kathy Johnston

Solidarity physicist is unable to gain scientific employment

London

DR Andrzej Jurewicz, a leading Polish particle physicist and a former Solidarity activist, has now been without a job for four years, according to a recent newsletter of the unofficial Polish "Social Committee for Science" (SKN). This committee, whose membership is not announced but which is believed to include several members of the Polish Academy of Sciences, acts as a human rights watchdog for the scientific professions, as well as sponsoring bursaries for scholars unable to complete their research within the official scientific establishment.

Jurewicz formerly worked at the Institute of Nuclear Research at Swierk, outside Warsaw, where he headed the particle physics group, and, during 1980-81, was head of the working party for scientific affairs in the institute's Solidarity chapter. At the end of 1982, the institute was 'reorganized' into three smaller institutes, and as a result the authorities were able to dismiss known Solidarity supporters without contravening Poland's new labour laws. Valuable

research was thus abruptly terminated, and the dismissals were crudely carried out, with those dismissed being refused entry to the institute either to clear their desks or to collect their severance pay (see *Nature* 303, 565; 1983).

Shortly before the "reorganization" of the institute, Jurewicz had been recommended by his colleagues for a full professorship. Since 1 May 1983, Jurewicz has been officially "at the disposal of the State Atomic Energy Agency", but has been consistently blocked from obtaining a post either in the agency or otherwise. During the past four years he has been employed for just four months.

Jurewicz is now the only former member of the Department of Theoretical Nuclear Physics at the disbanded institute not to have been given scientific employment. He has attempted to bring a case through the Labour Court, but on 30 April 1986 the court rejected his plea and sent the files on his case to the PAA for consideration. So far, said the SKN, in its "Information Service" bulletin dated 5 April 1987, "the president of the agency has not replied".

Vera Rich

Disinformation and fraud

SIR—As one deeply concerned with the nature of information in Western societies, I was distressed at the prejudicial framework in which you presented the Stewart/Feder study (15 January 1987). Although I am not competent to judge the scientific issues, I am qualified to discuss the presentation of information. In that respect, I think you are remiss.

Your leading article and the articles by Stewart/Feder and Braunwald raise the following questions:

How are we to know that the Stewart and Feder work "...is not itself above reproach..." when, as you note, you are not publishing the earlier complete version?

Is not your presentation biased against Stewart and Feder because of the trouble their study and their allegations of scientific fraud have caused you? That is certainly the impression one receives from your description of the tortuous process by which the article was published.

Certainly an editor has the right, if not the duty, to discuss whatever issues he deems fit in an editorial column, but weak sarcasm such as "thanks to the marvellous powers of word-processors..." the use of negative words and phrases such as "tedious details," "injudiciously" as well as "the truth is that the document by Stewart and Feder has now acquired a notoriety comparable with the Darsee affair itself" characterize Stewart and Feder as obnoxious children intent on being heard at all costs.

How, indeed, can those who discuss disinformation be as notorious as those who perpetrate fraud? Are "honest mistakes" and minor discrepancies to be tolerated? One need only consider the Challenger disaster for an answer to that question. Yet the pious tone of your presentation, and that of Braunwald's, obscures the impact of the Stewart/Feder findings by mildly agreeing that they were probably right in some areas but are making too much of it. How can anyone make too much of such errors, inaccuracies and misleading statements in a world poised on the brink of so many disasters?

In particular, Braunwald utilizes intellectual sleight-of-hand to answer accusations with accusations, as on the co-authorship issue. Either he was involved and should have known what was happening or he was not and should not have been a co-author. He cannot have it both ways while denying Stewart and Feder the right to have it either way. To quote Braunwald, "how is the cause of science served by making such unfair accusations?"

Is not your editorial remiss in ignoring the fact that Braunwald, whose comments in response to Stewart/Feder are endorsed in your editorial, was one of Darsee's co-

authors? Only by reading Braunwald's commentary does one discover that he is not an objective commentator. Further, nowhere in your issue of 15 January may readers learn that Braunwald's attorney was responsible for a considerable portion of the litigious threats that led to the current abridgment.

One might also wonder about your uncited reference to the *New York Times* (26 April 1986)... as that edition carries no reference to this case. If your reference is to the *Times* of 22 April 1986, one might overlook your honest mistake of dates but your phrase "...their ...series of statements..." in no way fairly or accurately reflects Philip Boffey's lengthy and well-balanced feature article.

Perhaps these items are minor and insignificant. But given the nature of information, it seems incumbent on every publication to present information carefully and without prejudice.

Neither should one assume, *a priori* that published information is correct. One need only look at cases such as Darsee's, that of Drs Stalcup and Mellin at the Columbia College of Physicians, Bio-Tech Lab's 208 fraudulent studies, how the scientific community reacted to Immanuel Velikovsky, Derek Freeman's indictment of Margaret Mead's work, the implications of the Broad/Wade book, *Betrayers of the Truth*, Einbinder's *The Myth of the Britannica* and to numerous other examples of disinformation to know that our information systems, at best, are pretty tenuous.

At the very least, it seems incumbent upon publications such as yours to approach controversy with the open spirit of the empirical method. Let the data speak and avoid pious, prejudicial characterizations. Unfortunately you have not done so in this case.

DAN GREENBERG

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• Readers must judge for themselves on most of the points raised by Mr Greenberg, but he is mistaken to imply that the original version of the Stewart/Feder article would have been published (at nearly twice the length) had it not been for the intervention of Dr Eugene Braunwald's attorney.

Editors have a duty to potential complainants (as well as to their colleagues, whose salaries may be at stake) not to publish libels. The libellous character of the original version was self-evident, an opinion confirmed by *Nature's* own legal advisers and, later, by Braunwald's attorney; plainly it is not an easy task to ensure that statements based on inferences from a scrutiny of the scientific literature, and

purporting often to describe the state of mind of those concerned, should be capable of substantiation. (In US libel law, defamatory falsehoods are vulnerable; in English law, under which truth is not a sufficient defence, the attribution of malign motives to people is a familiar recipe for aggravating jury damages.)

The chief abbreviation of the Stewart/Feder manuscript (with the consent of the authors) was the omission of a long and speculative discussion of the reasons why researchers sometimes publish lies and carry co-authors with them in the process; from his personal knowledge of the texts, Greenberg must know that his suspicion that the full text would have been beyond reproach is misplaced.

Many but not all of the later comments on the truncated text offered by Braunwald's attorney were acknowledged by *Nature* to bear on statements whose veracity could not have been substantiated; on most but not all of these occasions, the authors agreed, not always reluctantly, that their text should be amended accordingly. The value of what Stewart and Feder have done is to draw attention to a serious problem in the organization of research and its reporting in the scientific literature which would not, in the opinion of *Nature*, have been enhanced if it had been libellous as well. — Editor, *Nature*.

SIR—Stewart and Feder's investigation of a scientific fraud (*Nature* 324, 207; 1986) left me dissatisfied because it did not mention the role of journal editors in publishing fraudulent or inferior material. Clearly the editors are responsible if such material is published. Some of the discrepancies in some of Darsee's publications, for instance, were quite blatant, and should have been noticed by a reviewer. It also seems that the inclusion of a leading figure among the authors helps to get an article published.

I hope the Stewart and Feder article will teach editors the lesson that thorough reviewing is important, and that quality and not authorial prestige should decide what gets published.

PETER P. HEILEMANN

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Unemployment figures

SIR—On behalf of the Save Science Society, I am making a survey of unemployment among British scientists and would like to hear (in confidence) from those affected, with brief details of professional qualifications, areas of previous study, publications and previous employment.

BARBARA HALL

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Superconductivity theories narrow down

While the explanation of ceramic superconductivity remains elusive, new data serve to eliminate conventional strong coupling between electron pairs and phonons.

THE discovery¹ of superconductivity in Cu–O perovskite-type materials with transition temperatures from 30 to 100 K has raised the question of the dynamical mechanism responsible. Superconductivity in metallic systems has hitherto invariably been caused by an attractive interaction, mediated by the exchange of lattice vibrational excitations (phonons), between electrons forming coherent pairs in the superconducting state².

That phonons are essential for the understanding of conventional superconductivity was clear in 1950, with the discovery of the 'isotope effect'^{3,4}. The substitution of a different isotope for the positive ions in a metal was found to change the transition temperature by about one-half the percentage change of mass. The isotope effect was accounted for quantitatively in 1957, when the interaction in the fundamental BCS theory⁵ of superconductivity was interpreted as arising from the phonon mechanism.

A recent experiment by Batlogg *et al.*⁶ at AT&T Bell Laboratories now seems effectively to have eliminated the phonon mechanism in the high- T_c (90 K) superconductors $\text{YBa}_2\text{Cu}_3\text{O}_7$ and $\text{EuBa}_2\text{Cu}_3\text{O}_7$. By replacing 75% of the usual ^{16}O isotope by ^{18}O , they have found the Cu–O stretching frequency to change by the expected 4%, but the change in T_c is less than 0.25% — only about 0.3% of T_c . Although the isotope effect can exceptionally be less than the effect on phonon frequencies, it is unthinkable that the isotope effect can be 5% of that expected if the superconductivity is driven by phonon exchange.

As Sir Nevill Mott recently pointed out⁷, there are almost as many theories of these superconductors as theorists. This result will eliminate many, but otherwise is not unexpected^{7,10}. When transition temperatures above 15 K were first found, people asked whether the phonon mechanism could produce still higher T_c s and concluded^{11–14} that values 30 K were not to be expected. Such arguments have recently been applied^{8,9} to the newer materials: a generous estimate gives an upper limit of 40 K.

At least two experimental results had already indicated that conventional phonon pairing could not be responsible. Neutron diffraction measurements^{15–18} in La_2CuO_4 , which is antiferromagnetic but not superconducting, show atomic moments on Cu sites of almost 0.5 Bohr magneton. Magnetism in the d -transition series to which Cu belongs is invariably a result of strong interelectronic repulsive forces¹⁹ — especially in insulating transition metal-oxides such as La_2CuO_4 . Yet the material becomes superconducting when 10% of the La is replaced by, for

example, Sr.

Unless there is a complete change in the fundamental electronic structure between the crystallographically identical La_2CuO_4 and $\text{La}_{1.8}\text{Sr}_{0.2}\text{CuO}_4$, no theory based simply on attractive forces between opposite-spin electrons is likely to be correct. So strong-coupling phonon mechanisms as in conventional BCS theory, as well as the single-site bipolaron mechanism⁶ and other proposals which are conventional BCS theories with unconventional pairing are eliminated. But bipolaron mechanisms, whether single-site or not, driven as they are by electron–phonon coupling, are also now effectively eliminated by the absence of the isotope effect.

We argue that the antiferromagnetism of the parent compound indicates strong short-range interelectronic repulsion which must be taken into account when constructing a pairing theory for these superconductors. This force occurs in the heavy-fermion compounds which exhibit superconductivity and antiferromagnetism (at low temperatures). There, the pairing appears to be driven solely by electron–electron interaction and the effect of repulsion is to make the pairing anisotropic, an effect expected in single crystals of the new materials. In this framework, exchange mechanisms consistent with the repulsion, such as antiferromagnetic spin fluctuations¹⁰ or localized charge transfer excitations⁷, are not excluded. Nor is the resonating valence bond (RVB) theory⁶, based entirely on short-range electron–electron repulsion.

The other set of data arguing against strong phonon coupling are those of Fleming *et al.*²⁰ and a number of other groups²¹ on the high-temperature (300–500 K) crystallographic (tetragonal to orthorhombic) transition in the lanthanum compounds. Because the Fermi surface of these materials must surely 'nest'²² very effectively, one would expect that, if there is strong electron–phonon coupling, the crystallographic transition would be accompanied by the appearance of an electronic charge density wave. But the crystallographic distortion that does occur has no effect whatever on the Fermi surface and appears to be almost irrelevant to the electronic structure. A mysterious force (the Princeton group actually feels it is not so mysterious²³) prevents what electron–phonon coupling is present from being very effective.

Some experiments have not received the ballyhoo which seems now to accompany new results in this field — the

observation of finite and rather large specific heats at low temperatures, suggestive of those of metals^{24–28}. All the new materials studied so far, including the parent non-metallic La_2CuO_4 , have this property. The oldest result of the quantum theory of metals is that the specific heat is given by γT , in which γ is the density of electron states near the Fermi surface. In superconductors, γ is characteristically absent because of the well-known BCS energy gap²; it is also very small, if not zero, in all insulators, again because an energy gap causes the absence of electron states at low excitation energy. But insulating La_2CuO_4 , superconducting $(\text{La},\text{Sr})_2\text{CuO}_4$ and superconducting $\text{YBa}_2\text{Cu}_3\text{O}_7$, all have specific heats which are linearly dependent on temperature and which are greater than the equivalent molar amount of copper metal¹⁹.

This linear behaviour in the superconducting samples, but not its magnitude, could be explained if the materials were 'gapless', but there is no other evidence of this. It is hard to avoid the conclusion that this pervasive behaviour of the specific heat is related to the superconductivity. This seems to eliminate most conventional theories and some unconventional versions of BCS. The linear behaviour is, on the other hand, predicted by the RVB theory of Anderson and collaborators⁷ and this observation gives their ideas support.

P.W.Anderson & Elihu Abrahams

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Plant stress

Adding ethylene to injury

Mike Unsworth

OZONE is a gas about which environmentalists are equivocal. Most atmospheric ozone is formed and resides in the stratosphere (12–40 km) where it is generally agreed to be beneficial because it absorbs otherwise harmful ultraviolet light. Consequently, substances that destroy stratospheric ozone, such as chlorofluorocarbons used in refrigerators and aerosol sprays, are viewed with concern. But ozone is also formed in the lower atmosphere from reactions involving pollutants such as nitrogen oxides and hydrocarbons. In hot, sunny weather these reactions proceed rapidly and, if there is little wind, ozone concentrations can become large enough to damage sensitive plants. It is difficult to assess the importance of this ozone as a plant stress because it often occurs simultaneously with other stresses such as drought. On page 417 of this issue, Mehlhorn and Wellburn propose that ethylene, known to be emitted from stressed plants, determines the susceptibility of plants to injury by ozone. If they are correct, this could be an important unifying concept for explaining ozone/stress interactions.

Plants produce ethylene, an important growth regulator, during normal metabolism. Ethylene production increases rapidly following trauma caused by chemicals, temperature extremes, drought, disease and insect damage. This enhanced production of 'stress ethylene' can accelerate the abscission of leaves or fruit damaged by stresses. Stress ethylene has often been used as a measure of ozone injury, but it does not seem to have been suggested previously that ethylene biosynthesis is a necessary condition for ozone injury to occur.

Mehlhorn and Wellburn find that leaves of pea seedlings grown for three weeks in clean air are severely damaged by only one exposure to ozone, and release large amounts of stress ethylene. In contrast, seedlings exposed every day to ozone produce very little ethylene and have no leaf injury after three weeks. To test the hypothesis that rates of ethylene emission influence the amount of leaf injury caused by ozone, the authors sprayed seedlings grown in clean air with an inhibitor of ethylene biosynthesis and exposed them to ozone. Compared with unsprayed controls, the seedlings that could not synthesize ethylene had substantially less injury. Finally, to test whether exposure to a second pollutant could increase stress ethylene production and so increase the ozone-mediated injury, they exposed seedlings to nitric oxide (NO) and

nitrogen dioxide (NO₂) as well as ozone. In each case, injury was greater than in ozone alone, and there was more ethylene emitted. Interestingly, exposure to NO or NO₂ without ozone did not lead to injury, but did increase ethylene emissions, and the authors speculate that the plants would therefore be susceptible to subsequent ozone exposure.

These results led Mehlhorn and Wellburn to suggest that plants are more susceptible to ozone injury if high ozone concentrations (or other stressing factors)

occur infrequently, because the capability for stress-ethylene production would be greater in such plants than in plants that had been continually stressed. Ozone undoubtedly does occur in this episodic fashion, and if this mechanism turns out to be generally correct, the value of the long series of growth experiments in which crops are exposed every day to ozone (Heck W.W. *et al. J. Air Poll. Control Ass.* **32**, 353–361; 1982) becomes decidedly dubious. But other evidence makes the case unproven at this stage.

Although it is possible that ethylene emission is no more than an indicator of biochemical processes that are disrupted by ozone, Mehlhorn and Wellburn favour the more exciting interpretation — that reaction between ozone and stress ethylene causes the observed injury. P.M

Survey of UK ozone distributions

THE figure displays the daily maximum ozone concentrations (hourly mean values) recorded in 1978 in Devilla forest, Scotland. Also shown diagrammatically and tentatively are contributions from major ozone sources, as reviewed in a recent report*. Prolonged exposure to levels above 50 parts per billion (p.p.b.) damages many species of trees and plants, whereas short-lived episodes of concentrations above 100 p.p.b. are also deleterious (see the accompanying article by Mike Unsworth). There seems to be a rising trend in tropospheric ozone levels around the globe but a lack of consistency between experimental monitoring stations renders it hard to arrive at a definitive verdict.

A principal source of ozone in the lower atmosphere is the photolysis of nitrogen dioxide by solar ultraviolet radiation, although downward transport from the stratosphere can be at least as important. A balance is struck as ozone reacts with the photolytically generated nitric oxide or is taken up at the surface. But other agents such as photochemically degraded hydrocarbons can also oxidize nitric oxide and thereby leave ozone levels enhanced.

The report provides a timely survey of ozone distributions in the United Kingdom and of the means by which people are try-

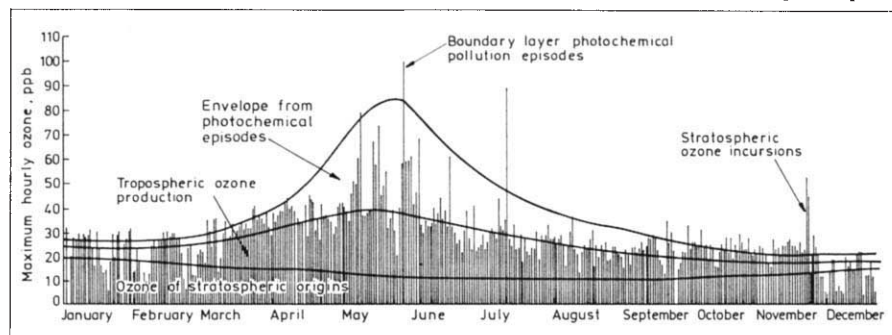
ing to account for them, including a striking (if idealized) depiction of the interlinked roles of meteorological and man-made chemical processes in an imaginary parcel of air passing over an isolated town in the early daytime.

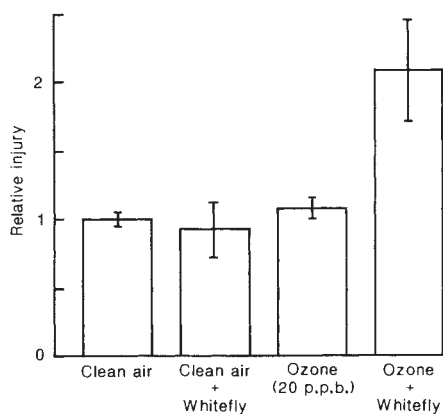
The parcel picks up nitric oxide and hydrocarbons which are mixed within the lowest kilometres by surface heating and turbulence. Within an hour the most reactive hydrocarbons (olefins) are photochemically oxidized and, in the presence of nitrogen oxides, start to enhance ozone levels as outlined above. The parcel moves away from the town and ozone levels increase over the next 10 hours or so, as aromatic hydrocarbons followed by olefins of medium reactivity make their presence felt. Ozone production declines after sunset, but the chemical destruction or deposition of the gas is hindered, if the circumstances are particularly unfavourable, by its being trapped above a nocturnal temperature inversion layer. On subsequent days the hydrocarbon-associated processes continue so that ozone levels only start to decline once hydrocarbon oxidation is complete several days later.

Such an episode is particularly likely to occur in summer, when typically low wind speeds allow the build up of precursor chemicals while reaction rates are enhanced by sunlight and by higher temperatures.

Philip Campbell

*Ozone in the United Kingdom by the UK photochemical oxidant review group, available from Department of the Environment, Victoria Road, South Ruislip HA4 0NZ, UK, price £10.





Leaf injury on bush bean (*Phaseolus vulgaris* L. cv. Pure Gold Wax) exposed to ozone and whitefly (redrawn from the data of Rosen and Runeckles). Injury, shown here relative to clean-air controls, was measured by a spectral reflectance method sensitive to chlorosis.

Rosen and V.C. Runeckles have similarly speculated to explain injury of bean leaves exposed to ozone and whitefly infestation (*Env. Conserv.* **3**, 70–71; 1976; see figure) but they did not measure ethylene directly, nor did they try the elegant inhibitor experiment reported in this issue. The success of the ethylene inhibitor in suppressing foliar injury by ozone brings to mind the enthusiasm a few years ago for ethylene-diurea as a protectant from ozone injury (Carnahan, J.E., Jenner, E.L. & Wat, E.K.W. *Phytopathology* **68**, 1225–1229; 1978).

The hypothesis that ozone and ethylene react together to cause leaf injury is attractive. The gases are known to react rapidly to produce free radicals and release excess energy as light (several commercial gas analysers measure ozone by detecting light from the ozone-ethylene reaction). Free radicals take part in peroxidation reactions with biological materials. It is sometimes stated that peroxidation of lipids in cell membranes is the basis for ozone toxicity, but Mudd (in *Effects of Gaseous Air Pollution in Agriculture and Horticulture* (eds Unsworth, M.H. & Ormrod, D.P.) 189–203, Butterworths, London, 1982) argued that ozone is more likely to attack the proteins of cell membranes and to pass through membranes to undergo reactions in the cytoplasm. Perhaps the products of the ozone-ethylene reaction preferentially attack sites other than these favoured by ozone, causing more widespread cellular damage. It is also interesting to consider where the ozone-ethylene reaction takes place — inside the leaf or in the air surrounding it?

But attractive as the hypothesis seems, there are weaknesses that still need to be addressed. The most obvious is to ask whether the treatments used by Mehlhorn and Wellburn alter stomatal responses so that the amount and rates of ozone entering leaves differ between treatments. There is by no means complete agreement

that ozone uptake is correlated with foliar injury (Taylor, G.E. Tingey, D.T. & Ratsch, H.C. *Oecologia* **53**, 179–186; 1982), but clearly entry of a pollutant into the leaf is a prerequisite for injury to occur. Another apparent inconsistency is that a large release of stress ethylene is observed when plants grown in clean air are exposed for the first time to ozone, showing that ozone undoubtedly induces stress irrespective of subsequent ozone-ethylene reactions. It is interesting to speculate that exposure to ozone alone alters growth or development, whereas exposure to the products of the ozone-ethylene reactions might induce visible injury. I wish that Mehlhorn and Wellburn had continued their experiments for long enough to identify effects on growth. There is certainly plenty of evidence that rating plants for ozone sensitivity on the basis of susceptibility to foliar injury does not correlate well with assessments based

on growth responses (Heck, W.W. Heagle, A.S. & Shriner, D.A. in *Air Pollution* (ed. Stern, A.C.) Vol. 6 Academic, London, 1986).

Although there are still unanswered questions about the physiological responses of plants in the ozone-ethylene experiments, the approach is sufficiently promising to suggest that the research groups studying ozone-stress interactions will be brushing up on their gas-chromatography techniques and ethylene biochemistry. It remains to be seen whether this observation clears a path through the minefield of pollutant-stress interactions or whether it is yet another example of the complexity and variation of responses that makes understanding these interactions such a difficult challenge. □

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Tropical health

DNA probe for river blindness

David Bradley

THE major parasitic infections of man, graphically called by the French *les grands endemies*, often vary markedly in their clinical manifestations and severity from one region to another. This is true of onchocerciasis, the filarial infection of man transmitted by blackflies of the genus *Simulium* and causing river blindness in Africa and Latin America. Regional differences in parasitic disease can be caused by variations in the host, in the parasite or in the pattern of host-parasite contact. New molecular-genetic data on the river-blindness parasite, reported by Erttmann *et al.* on page 415 of this issue¹, distinguish between two strains of the parasite and will help to control the disease.

Because the pattern of host-parasite contact is universal, difficult to measure, and is an obvious major determinant of variation (those who are exposed to many infective bites daily throughout life are clearly more likely to be heavily infected than the passing tourist who gets bitten only a few times), the role of host and parasite variation is hard to assess. Nevertheless, it has long been believed that this is not the whole story and that strains of *Onchocerca volvulus* worms that cause river blindness vary in their pathogenicity². The high prevalence of blindness in the savannah regions of Africa has been contrasted with its relative scarcity among infected populations in the forest zone further south, even when people with similar numbers of larval filariae in their skin are compared. There are differences in the pathogenesis^{3,4} and in the microfilariae⁵, but none of them can distinguish

effectively between strains. The paper in this issue¹ by Erttmann and his colleagues from four continents is therefore of particular interest. It shows a clearly marked difference in the DNA of the forest and savannah strains.

Specifically, Erttmann *et al.* find that a DNA probe, pFS-1, comprising 153 nucleotides from a forest strain (admittedly selected from 15,000 colonies from a library in a plasmid vector) hybridizes with a large DNA fragment in preparations from four other isolates from the forest zone and one from an intermediate zone, but hybridizes with neither of two savannah strains nor with various other DNAs — most importantly not with DNA from man or the *Simulium* vector.

Clearly, more parasite strains must be tested to be sure of the general validity of the probe. Nevertheless, on present evidence the forest and savannah strains of *Onchocerca* differ in at least one important genetic respect. We now have a marker, but so far it tells us nothing about how *Onchocerca* causes disease. For this purpose, probes from the savannah form, representing genetic material missing from the less pathogenic forest strains, might be more useful.

In the field, however, the forest-strain probe pFS-1 is of greater immediate practical importance. For the past 12 years a massive onchocerciasis control programme has been mounted in the savannah zone of West Africa⁷. It involves seven African countries and almost as many international organizations, and work during the first decade cost more

than 75 million dollars. The programme aims to stop onchocerciasis transmission over an area of 750,000 km² in which about 1.5 million people are affected and 120,000 blind, and thus will make socioeconomic development feasible. The main weapon, in the absence of safe, effective drugs to kill the adult worms, has been to kill the *Simulium* vector (biting fly larvae) in the streams where they develop, initially with the organophosphorus insecticide temephos (Abate), and to spray the toxic *Bacillus thuringiensis* from the air.

Remarkable success has been achieved in preventing blackfly breeding and halting transmission: vast areas have been cleared of the vector and, in man, the worms are dying out. But repopulation with blackflies that have flown or drifted (sometimes more than 100 km) is a problem⁷. Looking ahead to when the *Onchocerca* has largely died out from the area of the programme, there is the additional hazard of reinvasion by *Onchocerca* strains from outside. Should this occur, the ability to identify positively which

introduced worms were of the less pathogenic forest strain would be of the utmost importance. The pFS-1 probe will identify a single worm as of the forest strain, and may even be able to do this using a micro-filaria from the skin. Thus, the latest findings of molecular genetics can aid the field epidemiologist in the arid savannah in preventing blindness among the rural poor. The results exemplify the importance of scientific progress for tropical health — but field programmes are needed so the discoveries can be used. □

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Developmental neurobiology

Specificity of cerebellar grafts

Stephen B. Dunnett

WIDESPREAD interest in the functional potential of transplanted nerve cells has recently been generated by the report of a dramatic improvement in symptoms in two parkinsonian patients who received grafts of adrenal medullary cells into the brain ventricles. This result raises the issue of the mechanism by which the grafts exert their functional effects. Do they simply secrete neurohormones or growth factors into the brain, as has been suggested to account for the extent of success in the recent clinical trials (see the News and Views article¹ by S. Snyder), or can the grafts actually become connected into the host brain circuitry? If the fibre connections between the grafts and the host brain are important for the functional capacity of the grafts, is the growth of these connections guided by distinct regenerative processes or, in the case of embryonic cells, do they follow a normal developmental programme? On page 421 of this issue², Sotelo and Alvarado-Mallart present perhaps the most elegant data yet to indicate that grafted cells can reform very precise and appropriate connections with and from the host brain, and that the time-course of the growth of these connections mimics very closely the sequence of events in normal development.

The model system chosen by Sotelo and Alvarado-Mallart is the growth of the giant Purkinje cells in the embryonic cerebellum grafted to the cerebellum of a mutant (*pcd*) strain of mice in which the

Purkinje cells degenerate in the first weeks of life. The beauty of this model is that the normal cerebellum is organized in a regular laminar arrangement, with inputs from various cell groups making synaptic connections at precise sites on the dendritic tree of Purkinje cells. These authors have previously described how grafted Purkinje cells migrate into the molecular layer of the host cerebellum, replacing the missing neurons in the *pcd* host, where the cells then form dendritic trees appropriately organized perpendicular to the parallel fibres in the cerebellar cortex, and the precision with which the different input fibres make structurally normal connections at the appropriate locations on the dendrites³.

In their new report², Sotelo and Alvarado-Mallart followed the time-course of the migration and connectivity of grafted Purkinje cells, which turns out to be remarkably similar to the detailed timetable of normal development *in situ*. Thus, cerebellar primordium taken from embryonic donors at the twelfth day of gestation becomes integrated with the *pcd* host cerebellum 4 days later, at which time Purkinje cells begin migrating from the graft into the proper domain of the host cerebellum. This outflow reaches a maximum rate 2–3 days later and migration stops within 7 days, indicating that the attractive influences governing migration are present even in the adult cerebellum but that the migratory potential of the

developing cells wanes at a particular developmental stage both *in situ* and after transplantation. Following the period of radial migration, the grafted Purkinje cells develop monopolar spiny dendrites, on which parallel fibres and basket and stellate interneurons form synaptic connections at the appropriate locations. Synaptogenesis does not begin until 15 days after transplantation and is virtually complete within 21 days. Again, the cells and axons of the host retain the capacity for synaptogenesis into adulthood, but these connections are formed only within the time sequence determined by the grafted embryonic Purkinje cells in line with normal ontogeny.

How do these observations relate to other studies on neural transplantations as a means to reconstruct brain damage? Sotelo himself has provided a provocative challenge to other graft studies: he has distinguished between 'global' systems (diffusely projecting, regulatory pathways) and 'point-to-point' systems (precise cell-to-cell connections in pathways involved in transfer of specific patterned information), and argues that grafts of the former type exert their functional effects by a simple paracrine mechanism involving diffuse secretion of deficient neurochemicals into appropriate terminal areas within the host brain, whether or not fibre outgrowth and synaptic connections are formed³. By contrast, he argues, functional recovery following transplantation in point-to-point systems requires a much more exact morphological reconstruction involving precise regulation of the migration and the formation of afferent and efferent synaptic connections of grafted cells³. The cerebellar grafts in *pcd* mice then provide a demonstration that this level of precise morphological reconstruction can take place in appropriate model systems^{2,3}.

With global system transplants, such as dopaminergic or cholinergic neurons where functional recovery is well known⁴, both the growth of graft–host connections and the functional changes can take many months to develop fully. Therefore, the formation of synaptic connections with appropriate targets cannot be under the precise developmental control suggested for the Purkinje cells, which provide a prototype point-to-point system. What remains unknown is whether the cerebellar grafts can exert any ameliorative effect on the motor ataxia in the *pcd* mutant mice. The precise developmental regulation of synaptic connectivity seen by Sotelo and Alvarado-Mallart² implies that any behavioural effects would become fully apparent within 3 weeks, rather than developing over months as is generally the case for grafted cells of the global type. Other model systems have been described that might lay equal claim to point-to-point status, such as the relay of cortical

information to pyramidal and extra-pyramidal motor systems through the neostriatum. Grafted striatal cells can reconstruct precise afferent and efferent connections with the host brain, and provide the basis for recovery in both spontaneous and complex behaviours⁵. Although the time-course of regrowth and functional recovery in animals with striatal grafts has not been studied as systematically as the present observations on cerebellar grafts in the *pcd* mouse, the full extent of striatal regeneration in adult hosts is extended over a period of months, rather than taking place within the restricted time-frame that applies to the ontogenetic development of these connections.

How can these different perspectives be resolved? There is growing realization that grafted neuronal cells can exert a functional influence over the host brain by various mechanisms, from the acute release of growth and trophic factors into the damaged host central nervous system, through the chronic secretion of deficient neurochemicals (the paracrine model) and the tonic reinnervation of the host brain, to reciprocal axonal growth and reformation of synaptic connections between the neurons of the graft and host

brain⁶. The extent to which a particular degree of appropriate structural and functional integration takes place depends not only on the model system, but also on the lesion and other growth-promoting events in the host brain, the developmental age, placement and constituents of the graft, and the particular set of connections under investigation. The identification and characterization of the factors influencing the extent of anatomical reconstruction and the mechanisms of function of neural grafts is a topic of active research interest in several laboratories. The observations by Sotelo and Alvarado-Mallart indicate the extent and precision of structural reconstruction that can take place under favourable circumstances. □

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Environmental ecology

Unforeseen effects of control

Malcolm Coe

IN our haste to reduce the effects of pests and diseases on the secondary production of our domestic stock, we are often less informed than we should be about the broader ecological consequences of such new methods of treatment and control. Wall and Strong, on page 418 of this issue¹, draw our attention to the potential dangers of using the anti-parasitic drug ivermectin, not because of its effect on the large herbivores themselves, but because of the much less obvious, but vitally important, detritivore community associated with their dung.

Ever since the first terrestrial herbivores evolved, their faeces have provided a valuable source of food for various invertebrates, many of which are dung specialists. Predominant among this diverse fauna are the 14,000 species of dung beetle (Family *Scarabaeidae*). These insects have evolved a high degree of sociality in the care of their eggs and larvae, which complete their development in dung or broodballs that can be located in the dung source or in an underground brood-chamber. These beetles, which range in mass from 19 g (*Heliocopris dilloni*) to 0.004 g (*Drepanocereus parallelus*)² are found throughout the world and are important removal agents of both herbivore and carnivore dung (see

figure); some species (for example, *Anachalcos convexus*) use dung and carrion as a food source for their own maturation and as nutrient provisions for their larvae. Although the beetle fauna of individual continents have evolved genera and species that specialize on the dung of endemic mammals, the genus *Onthophagus* is virtually cosmopolitan in its distribution³.

Because of the limitations imposed on their breeding by the climate and the somewhat impoverished large mammal fauna, the temperate dung-beetle faunas are far less diverse than their tropical counterparts. In the Tsavo national park, eastern Kenya, up to 48,000 beetles have been recorded visiting a 3 kg pile of



elephant dung in 2 h, during which time all the soft edible material had either been eaten *in situ*, rolled up to 220 m away from the deposit or buried below it. This rapid mode of removal underlines the ecological importance of coprophagous insects in incorporating this digested plant litter into the soil, where much of it is decomposed by bacteria and fungi and its nutrients released for future plant growth. In addition to simply removing dung from the immediate soil surface, the burrowing activity, especially in arid to semi-arid environments, is of considerable significance in soil aeration, enhanced water percolation and in elevating up to 4 kg of soil to the surface for every kilogram (fresh weight) of elephant dung deposited in East Africa⁴.

Australian biologists realized the significance of dung-beetle activity when they observed that their endemic species were only adapted to the pelleted dung of herbivorous marsupials and were incapable of removing the dung deposited by introduced cattle. This dung in consequence persisted on the ground for long periods, suppressing grass production and providing breeding foci for flies. Since the mid-1960s CSIRO entomologists have carried out an extensive research programme in which they have introduced a range of dung-beetle species from Africa and elsewhere. These species have established themselves in many areas and have acted as efficient agents of dung removal, considerably reducing seasonal fly populations⁵.

Wall and Strong in this issue¹ demonstrate that dung samples collected from calves fitted with rumenal boluses delivering ivermectin at 40 µg per kg per day contained a total of only 17 adult dung beetles, 35 dipterous larvae and 44 earthworms after 100 days, compared with controls, which yielded 780 dung beetles, 267 dipterous larvae and 46 earthworms over the same period. Although the authors correctly point out that these drug-induced effects may vary with the manner in which the drug is administered, as well as with the geographical location and the climate, the ecological side-effects of such control methods need extensive study. Such influences on the local dung fauna could be particularly significant in developing countries where drug administration may be less stringent and the potential effects of dung removal and nutrient cycling catastrophic. □

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Atmospheric physics

Strange new whistlers

Michael J. Rycroft

WHISTLERS are radiowaves of audio frequency whose source is an almost instantaneous lightning discharge. As well as propagating in the Earth-ionosphere waveguide at speeds slightly less than that of light ($\sim 0.95c$), these waves can penetrate the ionosphere. Such whistler-mode waves are guided through the magnetosphere from one hemisphere to the other by ducts of enhanced plasma density, which are aligned along geomagnetic field lines. On page 405 of this issue¹, Armstrong reports a strange new phenomenon — the apparent triggering of one whistler by an identical one occurring about five seconds earlier. Armstrong considers the likelihood of this type of event statistically and concludes that it is not a chance coincidence.

The dynamic spectra (frequency versus time spectrograms) of whistlers show that the lower-frequency components travel more slowly than the higher-frequency ones. When detected on a suitable radio receiver, they are heard as swooping whistles — hence the name 'whistler'. Whistler frequencies are less than the electron-plasma and cyclotron frequencies that characterize the plasma; in solid-state physics, whistlers are termed helicons.

In space physics, whistlers are analysed to estimate the plasma frequency, and hence the plasma density, at the apexes of the field lines with geocentric distances in the equatorial plane of up to several Earth radii². Whistlers can be amplified near the equatorial plane by cyclotron-resonance interactions with energetic electrons trapped in the magnetosphere. In such a wave-particle interaction, electrons spiralling along the field lines lose transverse

energy to the waves and their pitch angles are reduced, enabling them to penetrate further into the upper atmosphere. These 'precipitating' electrons become lost from the radiation belts, as described in my previous News and Views article³. Cyclotron resonance with very energetic electrons (> 50 keV) is favoured on the field line just beyond the plasmopause.

Apart from creating whistlers, thunderstorms also maintain the ionosphere at an equipotential of about +220,000 V with respect to the Earth⁴. Positive charges are transported upwards by the updraught above thunderclouds. Away from the thunderstorms, there is an electric field pointing downwards, the so-called 'fair-weather field' ~ 130 V m⁻¹ near the Earth's surface^{4,5}. However, within a thunderstorm⁶, the upwards electric field can reach $\sim 10^5$ V m⁻¹. Thus the troposphere, stratosphere, ionosphere and magnetosphere are intimately linked electrically, by the global electrical circuit⁴. This circuit can exhibit many strange behaviour patterns⁷ over a wide range of timescales⁸. A component of the circuit, critical because of its influence on the current flowing through the atmosphere, is the high resistance column over a thundercloud⁹.

Armstrong¹ shows that whistlers can be synchronized so that one can be followed 5 seconds later by another. A possible five-step mechanism (shown in the figure) involves the electrons that are precipitated after amplifying the half-hop whistler (as described above) on its southwards journey. As they enter the upper atmosphere, the electrons must reduce the resistance of the column above the thundercloud by ionization, triggering an upwards

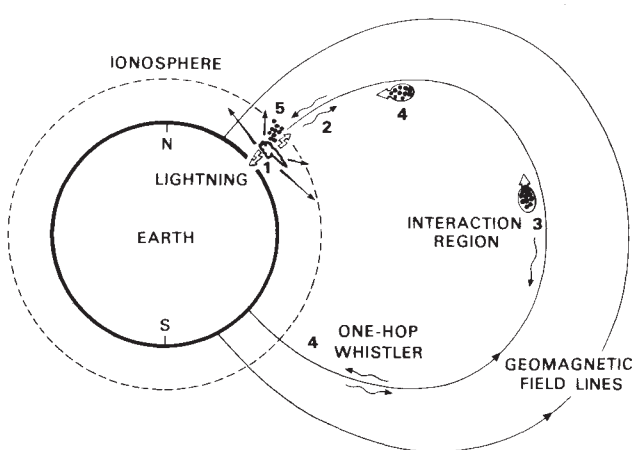
lightning discharge. It is this discharge that causes the second, synchronized whistler heard in the other hemisphere. But what is the precise physical mechanism by which the electrons have this effect?

First, there is the problem of energy. It is the more energetic electrons that penetrate lower into the atmosphere; even 100-keV electrons reach down only to about 80-km altitude, creating secondary ionization there⁹. But this is nowhere near the top of the thundercloud at a height of, say, 10 or 15 km. Such decelerating 100-keV electrons also produce bremsstrahlung X-ray radiation¹⁰, of less energy. This can travel downwards, and ionize the air down to 30 km. But is this X-ray flux significantly greater than the background flux resulting from cosmic rays? Bremsstrahlung X-rays are also produced by energetic electrons accelerated in the large electric field of a lightning discharge itself¹¹. Perhaps this process helps to initiate the upwards lightning discharge. Will the channel of reduced electrical resistance in the electric field of the stratosphere permit an upwards lightning discharge, and what would the characteristics of such a discharge be?

There is also a problem of timing. The 100-keV electrons are almost relativistic, and take only a fraction of a second to travel along a field line just outside the plasmopause from the equatorial plane to the top of the atmosphere. Therefore, the triggering process itself must include an inherent delay of a few seconds.

By appealing to results obtained when strong, localized electric fields are applied to a fluid or to a crystal, Armstrong argues¹ that a lightning discharge up to the ionosphere could occur as required. The next step is to pose some definitive questions, and to answer these with more research, both experimental and theoretical. It might be worth considering whether a contributory factor could be the behaviour of radiation-belt electrons trapped in the real geomagnetic field¹²⁻¹⁵, which differs appreciably from a centred-dipole model field in the meridian (longitude range) where these observations were made. It is also interesting to speculate as to whether whistler-mode signals

The five steps by which one whistler could trigger another. 1: A cloud-to-ground lightning discharge (north) radiates very-low-frequency radio-waves in all directions, as shown by the arrows. 2: Whistler-mode waves are launched into the magnetosphere. 3: A cyclotron resonance wave-particle interaction occurs near the equatorial plane. The southwards-going whistler is amplified and the northwards-going electrons have their pitch angles (between velocity and field vectors) reduced. 4: The one-hop whistler, received at Siple station in Antarctica, is reflected back into the magnetosphere. The bunch of electrons nears the end of the field line. 5: A two-hop whistler arrives in the north. The burst of precipitating electrons causes secondary ionization in the upper atmosphere. The resistance of the air column above the thundercloud is reduced, causing a lightning discharge to the ionosphere, and thus producing more whistlers.



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from man-made transmitters could influence lightning discharges.

Thus the outcome of this piece of research is in agreement with Robert Browning's sentiments: "How strange it seems, and new!" although Samuel Johnson held a different view: "All is strange, yet nothing new". It is fitting, in this

instance, that Lord Macaulay should have the final word: "His theory is therefore this, that God made the thunder, but that the lightning made itself". □

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Gene regulation

Why should DNA loop?

Robert Schleif

FOR its central functions of DNA storage and retrieval, DNA can be quite adequately approximated as a one-dimensional line of nucleotides. Recent experiments show, however, that this approximation will not suffice to explain transcriptional regulation. In some regulatory systems, the DNA loops out of one dimension to bring two specific well-separated DNA sites into close proximity (see figure). The evidence for looping in several prokaryotic systems¹⁻⁹ as well as one eukaryotic one¹⁰, has recently been reviewed in *Nature*¹¹. In all cases, sequences required for transcriptional regulation of a particular gene are found at a site more than 100 nucleotides away from the RNA-polymerase binding site, and a protein or proteins bound at this site is (are) believed to contact a protein or proteins bound close to the transcriptional start site. In the arabinose system⁴ and in an artificial construct involving λ phage repressor and operators *in vitro*^{12,13}, looping has been demonstrated between repressor molecules bound cooperatively at separated operator sites. Does this mechanism explain eukaryotic enhancer function? And if so, why might such a mechanism have evolved?

Enhancers, stretches of DNA perhaps 40 nucleotides long, are required for correct regulation of transcription of a gene. They were first identified in animal viruses, but have since been found to be

associated with many eukaryotic promoters. They can be located hundreds or sometimes thousands of nucleotides from the promoter and in many cases the major portion of the DNA in between is irrelevant to enhancer function. Before discussing the reasons for DNA looping, I will explain why it is that proteins which regulate transcription bind to DNA at all.

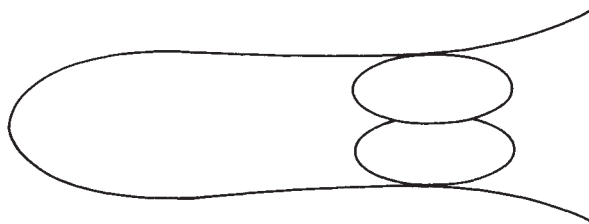
Regulating transcription of a gene requires that a protein sense the presence or absence of a suitable signal and communicate this status to the transcription machinery. To permit more than a handful of genes to be regulated simultaneously within a cell, the potential influence of a gene-regulating protein must be restricted to the appropriate gene. Therefore regulatory proteins are built to bind to DNA near a promoter so that they can modulate the activity of RNA polymerase(s) only at that promoter.

Two general reasons can be advanced for DNA loops. The first is geometrical. Only two or perhaps as many as four proteins may bind DNA next to and affect the activity of a protein such as RNA polymerase bound to a promoter. For a gene with a complex regulation pattern, how can more than two signals from proteins bound to DNA be sent to an RNA polymerase molecule? Looping is one solution, in which proteins next to a promoter-bound RNA polymerase molecule can modulate its activity, and proteins bound to DNA hundreds of nucleotides away can touch by looping to the adjacent proteins or RNA polymerase itself and also affect transcription initiation. Proteins may also be able to affect the looping and hence the regulation by binding in the middle of the loop at positions well away from the ends.

A second reason for looping is the cooperativity generated by the multiple site binding. For the purposes of this discussion, consider a single bivalent protein

that binds to two DNA sites and forms a DNA loop. This is merely an extreme of the general case in which two different proteins bind to the two sites and bind to each other to form the DNA loop. The cooperativity inherent in these cases derives from the fact that if the protein is bound to one site, its other DNA-binding region automatically is relatively near the second DNA site. That is, the presence of the protein on one binding site increases the local concentration of the binding protein to the other site and stimulates the binding of the protein to the second site.

The cooperativity resulting from loops can generate a high effective affinity of the protein for its binding sites. Consequently, only small amounts of a regulatory protein are necessary to occupy a DNA site almost completely. Such an effect may be essential in reducing the total necessary amounts of the thousands of regulatory proteins required to be within a cell or nucleus. In addition, the cooperativity that can generate nearly complete binding to DNA does so with sites whose intrinsic affinity is relatively



DNA looping promoted by proteins bound at two sites. If the two proteins, which can be identical or different, possess appreciable affinity for one another, they can hold the DNA in a looped state. Such looping facilitates gene regulation by permitting more than two proteins plus cofactors to affect transcription frequencies from a promoter by altering the binding or activity of RNA polymerase. The looping permits binding with low protein concentrations, facilitates rapid dissociation of each individual protein and, by virtue of the cooperativity inherent in such a system, generates nonlinearity that may be useful in gene regulation.

low. Consequently, the dissociation rates of the protein from either of the sites forming the loop will be high. Such a high dissociation rate may permit rapid transcriptional responses as well as facilitate, for example, the passage of DNA polymerase during DNA replication. Finally, the cooperativity might allow effective regulation to result from small alterations in the concentration of a regulatory protein. That is, a small decrease in the concentration of a regulatory protein could produce a large decrease in the occupancy of its binding sites.

Two points can be made with respect to the actual formation of loops that bring together domains of proteins bound to DNA. One extreme is to place the burden of looping on the DNA as implied in the above discussion. The opposite extreme is to let the protein do the looping. The interacting protein sites can be put on long flexible arms so that the appropriate domains can interact without much bending

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of the DNA. This may be the case for the yeast *HIS3* (ref. 14) and *GALI* (ref. 15) gene enhancers. The second point is that in some cases bending DNA into a loop may be energetically unfeasible without the intrinsic bending provided by supercoiled DNA. One such example is the arabinose system, which can loop *in vitro* only when the DNA is supercoiled³.

Not only does supercoiling help bring distant points together, but it can also assist formation of complex wrapped structures, such as occurs for the integration complex of the *int*, *xis* and *IHF* proteins of phage λ (ref. 16). □

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Climatology

Hundred-kiloyear cycle queried

Alan C. Mix

ONE of the major research issues of the past decade in palaeoclimatology has been to determine the cause of the 100-kyr cycle, which has seemed to dominate the apparent fluctuations of the ice ages preserved in the geological record. In a recent paper¹, Bill Ruddiman and Maureen Raymo of Lamont-Doherty Geological Observatory add a new twist to this picture. Their detailed study of hydraulic-piston-cores taken by the Deep-Sea Drilling Project confirms earlier suggestions² that the 100-kyr ice-age cycle is a recent aberration, prevalent only during the past 800 kyr.

Milankovitch hypothesized³ in 1941 that the Earth's climate changes in response to the changing geometry of the Earth's orbit. He predicted climatic cycles with periods of about 23 kyr (due to precession of the equinoxes, the circular wobble of the Earth's axis), 41 kyr (due to variations in the tilt of the Earth's axis) and 100 kyr and 400 kyr (due to the varying eccentricity of the orbit). Recently, palaeo-oceanographers studying microfossils from deep-sea sediments have developed timescales accurate enough to demonstrate convincingly⁴ the presence of these 'Milankovitch cycles' during the past several hundred thousand years.

The striking thing about this record is that the preserved 100-kyr ice-age cycle is the strongest, even though the direct eccentricity-forcing is the weakest of the orbital effects. In addition to their small, direct influence on collection of sunlight by the Earth, eccentricity variations modulate the strength of the precession effect, which controls where on the Earth's orbit the seasons occur. For example, when the Earth's orbit is perfectly circular it does not matter where summer or winter occurs. When the orbit is eccentric, however, the Earth-Sun distance varies throughout the year. Hotter summers would occur in the Northern Hemisphere when June occurred at a point closer to the Sun, as it did 11,000 years ago.

The 100-kyr climate cycle has generally been explained⁵ by the relatively slow growth and fast melting of large ice sheets. Through this nonlinear mechanism, many

believe that the ice sheets respond to the modulation of precession by eccentricity, and thus get their 100-kyr cycle indirectly. This theory relies on the internal characteristics of ice sheets, their long (and non-linear) time constants, to account for the observed 100-kyr cycle.

This theory is now questioned. First, strong 100-kyr climate cycles seem to have occurred before the late Cenozoic ice ages^{6,7}, implying that ice is not necessary to generate a 100-kyr rhythm. And second, the new evidence of Ruddiman and Raymo ironically suggests that the 100-kyr cycle is a rare occurrence for ice ages. This study confirms that the 100-kyr rhythm of glaciation appeared within the past million years, and became dominant only within the past half-million years. A much longer interval of the late Cenozoic ice ages, from ~2.4 to 0.8 Myr, was almost completely dominated by the 41-kyr tilt cycle.

The major question addressed by Ruddiman and Raymo is what caused the shift in rhythmic response of the ice ages to orbital forcing? They believe that evolution in character of the ice ages must result from some change in the external boundary conditions of climate (the configuration of land, sea, ice and atmosphere). They speculate that the rapid tectonic uplift of the Himalayas and parts of western North America during the past few million years has increased the sensitivity of the system, probably by inducing downstream meanders in the jet stream. These atmospheric waves would cause cold spots over eastern North America and Europe exactly where the large Northern Hemisphere ice sheets were located. Another theory, due to Pisias and Moore², suggests that the growth and decay times of smaller, land-based ice sheets of the early record were shorter than those of the more recent, larger, marine-based ice sheets. This might account for the growth of the 100-kyr cycle through time, but leaves open the question of why the more recent ice sheets were bigger than the earlier ones. Ruddiman's and Raymo's suggestion may solve this problem.

As noted by Ruddiman and Raymo, many uncertainties remain that will keep geologists and climatologists busy for years. First, of course, is the problem of developing timescales for geological sections accurate enough to read the cyclic patterns of climate change. Because Ruddiman and Raymo tuned part of their time scale to the 41-kyr tilt cycle, it is not surprising that the 41-kyr cycle dominated their time series. It is unlikely, however, that their tuning could have erased a 100-kyr cycle from the record.

Second, exactly when the major uplift of the Himalayas occurred and how fast it was is unclear. Although some believe⁸ that much of the uplift took place within the past few million years, others⁹ believe the Himalayas reached essentially full height more than 20 million years ago; details of the last 3 Myr are sketchy at best.

Third, how sensitive to topography are waves in the high-latitude wind fields? Different computer models give conflicting results for the cases of no-topography versus present-topography^{10,11}. No models have been run for intermediate cases of continuous uplift. Of course, the addition of the ice sheets adds topography to the system, and these boundary conditions have yet to be explored fully in general-circulation models of the atmosphere.

Finally, the observation of 100-kyr climate cycles before the ice ages^{6,7}, if correct, remains a mystery. If this rhythm is present during much of time before the onset of Northern Hemisphere glaciation, it may be that the interval of 41-kyr cycles from 2.4 Myr to 0.8 Myr found by Ruddiman and Raymo is the anomaly, and that the resumption of 100-kyr cycles at 0.8 Myr is simply the return to normal behaviour. At this point, there are more questions than answers. With the addition of long time series on climate change coming from the Ocean Drilling Program the rapid development of climate models, and the refinement of tectonic reconstructions, the next few years should see interesting developments (and more surprises) in this field. □

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Polymer science

Soluble conductors on the way

Paul Calvert

CONDUCTING polymers came to prominence in the mid-1970s, with many promising applications apparent, the most obvious being their use as cheap and light plastic conductors, organic replacements for copper. It soon became obvious that the materials available could not fulfill this role and interest in their use in solar cells, semiconductor devices and batteries grew instead. These now appear to be equally elusive. Four years ago I wrote a News and Views article¹ explaining why polymers could not be both soluble and conducting and, as a result, that the optimum way to form these materials was from a precursor polymer that could be readily cast into films or spun into fibres. Recent papers describing soluble polythiophenes^{2,3} prove me wrong.

The prototypical conducting polymer is polyacetylene (*a* in the figure), where the conjugated double bonds along the chain allow delocalization and mobility of electrons, but also lock the chain into a rigid zig-zag. Such a rigid structure gains little entropy on dissolution or melting, and so remains stubbornly solid until it decomposes. Polyacetylene itself is semiconducting but oxidation or reduction (doping) can remove or add electrons in the delocalized π -orbitals with the result that the polymer conducts like a metal. Polyphenylene (*b* in the figure) followed polyacetylene as the popular polymer to study and is also extremely insoluble, though the reasons are less clear, as the undoped state probably has little π -orbital overlap between the rings. Polyphenylene differs significantly from polyacetylene in this respect, as shifting all the double bonds sideways one 'step' in polyacetylene produces no change in structure or energy, whereas the same shift in polyphenylene, so that the rings are connected by double bonds, produces a quinoid structure which has a higher energy.

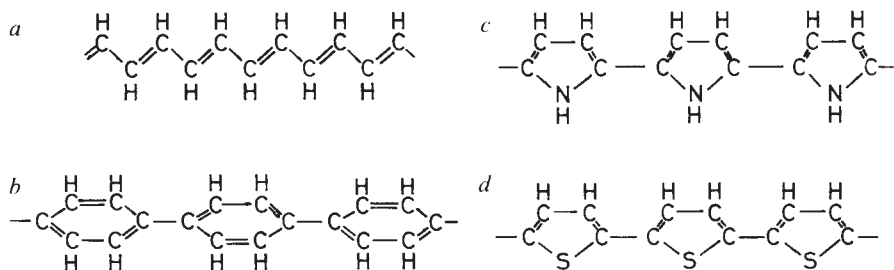
Polypyrrole (*c* in the figure) and polythiophene (*d*) also became the subject of intense interest and were equally insoluble. Both these polymers are related to a *trans*-*cisoid* form of polyacetylene. Thus it seemed clear that conduction requires a conjugated structure, but this in turn makes the chain rigid and insoluble.

Corrigendum

In the article by Noburu Miura on high-temperature superconducting ceramics (*Nature* 326, 638–639; 1987) Dr Jaw-Shen Tsai of NEC Corporation was incorrectly called Dr Aoi. Dr Hikami of Tokyo University was also incorrectly spelt as Dr Hikama. □

To the polymer scientist, this is a nuisance as the polymers cannot be purified or properly characterized.

There have been a few reports of soluble states. One method is to 'carry' the polyacetylene into solution by forming a block or graft copolymer with a soluble polymer such as polystyrene. The result, however, is essentially a micellar solution, the polystyrene surrounding the polyacetylene, and hence a low-conductivity state of polyacetylene^{4–6}. Polyphenylene-sulphide and polyacetylene form solutions when doped with arsenic pentafluoride in



Structures of four conducting polymers. *a*, Polyacetylene; *b*, polyphenylene; *c*, polypyrrole; *d*, polythiophene: *a* shows the zig-zag arrangement of carbon atoms found in *trans* structures; *c* and *d* show the crenulated arrangement found in *trans*-*cisoid* structures, seen by covering the NH or S.

arsenic trifluoride, but this is hardly a convenient solvent system for study and irreversible reactions occur with the dopant^{7,8}.

Work on polythiophene led to 3-methyl and 3-methoxythiophenes, which are also insoluble^{9,10}. The polythiophenes do have the advantage that they are relatively stable compared with polyacetylene and retain their conductivity over several months in air. A group at the Research Institute for Polymers and Textiles in Tsukuba now report² that 3-hexyl and 3-icosylthiophenes make soluble polymers that can be dissolved in the neutral or doped (and conducting) states. They can be doped to give conductivities of 5–100 S cm⁻¹, comparable to graphite. These polymers are formed by electrochemical polymerization. A group at Allied Chemical obtained similar results³ with studies of chemically polymerized thiophenes including 3-butyl and butyl-methyl copolymers. Poly-3-butylthiophene has a glass transition at 48 °C, making it a fairly rubbery polymer rather than a brittle, intractable structure as we have come to expect. The solubility allows molecular weights to be measured: these are rather low for real polymers but still quite respectable, with polymerization of 200–300 thiophene units. Clearly this is only the start; there are a mass of substituted thiophene and pyrrole polymers, copolymers and composites to work through before the limits of this class of materials are known.

At the recent American Chemical Society meeting in Denver (5–10 April), Alan Heeger of the University of California, Santa Barbara, described the spinning of fibres of soluble polythiophenes in blends with polystyrene or polyethylene oxide, and Herbert Naarman of BASF in Ludwigshafen described methods for drawing doped polyacetylene to give conductivities above 10⁵ S cm⁻¹, 100 times higher than usually found and comparable to copper. Heeger, Naarman and collaborators report their results on page 403 of this issue¹¹.

The surprising conclusion is that conductivities measured so far have been limited by the microstructure or perfection of the samples and the real limits may be higher still. The same could be applied to the low conductivities found for polythiophenes. M. Bryce and co-workers at

Durham University recently reported¹² an ether-substituted compound with conductivity greater than 1,000 S cm⁻¹.

It is easier to develop theories when the experimental answers are already known, and it should not be too difficult now to reconcile the twin requirements for conjugation and flexibility in these polymers. Since conducting polymers first excited interest, there have been many disappointments as various leads seemed to fade away. Now we are back with the original concept, a (more or less) stable, processible, film-forming, organic conductor. There is always a catch, but for now the field has a new dawn. □

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Muscular dystrophy

Mapping the disease phenotype

Miranda Robertson

DUCHENNE muscular dystrophy (DMD), a severe sex-linked degenerative disorder of muscle, affects about one male in 4,000, an estimated one-third of cases being caused by new mutations. The disease may (rarely) take a milder form called Becker muscular dystrophy (BMD); and either form can sometimes be associated with mental retardation. With the identification last year¹ of the gene responsible for DMD, it has become possible in principle to explain these characteristics of the disease at the molecular level, and a group of molecular and medical geneticists met recently* to review what is known, to attempt to formulate appropriate questions about what is not known, and to discuss the clinical implications of the emerging picture.

Structure of the gene

The most conspicuous feature of the *DMD* gene so far is its size: it spans at least 1,800 kilobases (kb) on the short arm of the X chromosome and neither its 5' nor its 3' end has yet been identified for certain. Many of the problems of both laboratory and clinic are directly attributable to the length of the gene. The transcript, for example, is an unmanageable 16 kb (ref. 1), only 20 per cent of which has been cloned and mapped to genomic DNA. This technique has revealed 20 exons of between 80 and 250 base pairs in length at the 5' end of the gene (Louis Kunkel, Boston Children's Hospital; Peter Ray, Hospital for Sick Children, Toronto).

With another 40 exons of about the same length now mapped, Kunkel and his colleagues Anthony Monaco and Michel Koenig expect the total to be about 100, if the same organization is maintained throughout the gene. Eric Hoffman in Kunkel's laboratory has compared the human and mouse *DMD* genes and finds the identified exons are conserved in sequence and size and probably also in organization between the two species.

A very high proportion of DMD and BMD mutations are deletions (indeed this was what originally made it possible to find the gene). Estimates of 11–12 per cent based on analyses using the published probes have now jumped to perhaps 50 per cent with the addition of data collected by Johan Den Dunnen using pulsed-field gel electrophoresis (Gert-Jan van Ommen, Leiden University). (This has important clinical implications which I

shall discuss later.) At the same time, a new probe (J-66) isolated by van Ommen and Ieke Ginjaar in Peter Pearson's group is the most 3' identified so far (together with one isolated by Corlee Bertelson, see ref. 3) and sets the present lower limit of 1,800 kb on the length of the *DMD* gene.

These data together immediately raise the following fundamental questions. When and where is the gene expressed? Are the exons differentially expressed in different tissues? Can the different disease phenotypes (Duchenne and Becker, with and without mental retardation) be explained by the differential deletion or disruption of specific exons? And what might the gene encode? None of these questions can be definitively answered by the existing data, but some possibilities can be excluded.

The very high-molecular-mass protein predicted from the long *DMD* transcript is not unusual for muscle proteins, and preliminary protein sequence data suggest a pattern of oligopeptide repeats that again is characteristic of α -helical coiled coil of muscle filament proteins such as myosin (Hoffman and Monaco, Kunkel's laboratory). The transcript is expressed in both fetal (Kunkel) and adult (Ray; Kunkel) skeletal and cardiac muscle, and probably also in smooth muscle, although this has been more difficult to establish.

Of the known muscle proteins, in many ways the most appealing candidate for the product of the *DMD* gene has been the suggestively named nebulin, a protein of the right order of relative molecular mass (about 500,000) and implicated in the structural maintenance of skeletal muscle^{3,4}. The pattern of expression of the *DMD* transcript, however, probably excludes it. Its principal advocate at the meeting was Massimo Zeviani (Columbia University), who reported that despite the difficulties of detection that have earned the protein its name he can see it on SDS gels from 40 controls, but finds it is faint or absent in 40 of 43 DMD patients. Nebulin, however, is notably absent from heart (ref. 4; Zeviani), while Kunkel and colleagues now report that the *DMD* transcript, contrary to their initial report¹, is present in mouse cardiac muscle.

There is so far no unequivocal evidence for either developmental-stage-specific or tissue-specific splicing of the *DMD* transcript. Ray reported 6 exons at the 5' end of a complementary DNA from adult muscle whereas Kunkel finds 4 in fetal complementary DNA; but these exons have been identified by hybridization alone and Kunkel believes the discrep-

ancy is as likely to be caused by differential use of restriction enzymes by the researchers as by differential use of exons by fetus and adult. Further analysis should quickly reveal whether the discrepancy is real or artefactual.

An alternative way of looking at the same question is to ask if deletions involving different exons produce discernibly different mutant phenotypes: specifically, can particular regions of the gene be implicated in the Duchenne, the Becker or the mentally retarded phenotypes? The short answer to this question is no. A longer one follows.

Towards the phenotype

A boy with Duchenne muscular dystrophy is in a wheelchair by the age of 12 and dead before he is 30. A boy with Becker may remain on his feet in old age and complete his normal span. Both DMD and BMD are variable in age of onset and severity, Becker more than Duchenne presumably simply because cases are not defined as Duchenne unless they are close to the upper limit of severity.

Participants at the meeting brought with them data from a total of 2,000 DMD and BMD families, and although not all of them had access to all the DNA probes it seems there is probably no simple relationship between either the location or the extent of the deletions in the gene and the severity of the phenotype. Possibly the most striking illustration of this point is a Becker patient still walking at the age of 61 with a deletion encompassing some of the smaller Duchenne deletions and spanning at least one third of the gene (Kay Davies, Oxford University).

This particular case is startling, especially given the very high amino-acid conservation between mouse and man in those exons sequenced to date; but it is not unprecedented for highly conserved proteins to be seriously disrupted without any obvious effect on phenotype: indeed there is a nematode mutant with a 10 per cent deletion in a gene encoding a muscle protein of comparable size to the *DMD* gene product but no detectable impairment of motility in normal conditions (Robert Waterston, personal communication). Nor is the general absence of a gross structural correlate of severity so surprising, because even the smallest deletion in a 5' exon could destroy the reading frame of all the remaining 14 or 15 kb, whereas a large deletion might cleanly remove a domain or two without unduly perturbing the tertiary structure of the protein.

What this means of course is that an understanding of the molecular basis of the DMD and BMD phenotypes requires detailed analysis of the individual mutations and ultimately the identification of the gene product and the elucidation of its function. As a beginning on the detailed analysis, Monaco reported preliminary

*The Molecular Biology of Duchenne Muscular Dystrophy, held under the auspices of the Association des Myopathes de France in Versailles, 8–10 April.

findings on three deletions involving those exons that have been cloned and sequenced.

One of these is a 6-kb deletion originally detected in a Becker patient by Kevin Hart (Guy's Hospital, London) and which results in the excision of one exon, probably leaving the adjacent exons and their splice junctions intact so that the rest of the coding sequence can be read in frame. The two Duchenne deletions that have been investigated by contrast can be shown to change the reading frame. The more distal end of the gene is so far relatively unexplored and may turn out to encode domains more critical for function than any of the 5' exons. Monaco and Kunkel are hoping that other researchers will be willing to supply them with further data on specific patients to follow up these analyses.

Although it is possible to see in broad outline how a combination of mapping, cloning and sequencing will eventually show how various different mutations in the gene encoding a muscle protein can give rise to a muscle disease of varying severity, the occasional association of DMD and BMD with mental retardation may prove more problematical.

First, there is no evidence so far that the gene is transcribed in brain. This, as Kunkel was careful to stress, may not mean anything: probes are only available for a quarter of the gene and it is possible that brain tissue expresses only or primarily those exons for which probes do not yet exist. On the other hand, if that is the case it should be possible to link the deletion of particular regions of the gene with the occurrence of mental retardation. No such association emerged from a collation of information at the meeting; but again, a pattern may emerge with data from an increasing number of probes.

In any case, it is perfectly possible for a gene to cause mental retardation without being expressed in brain at all. Such an indirect effect might be very difficult to

identify.

Finally, there is the problem of diagnosis. The consensus at the meeting seemed to be that mental retardation associated with DMD is a distinctive phenotype that breeds true within a family. But several participants quoted apparent exceptions to this rule and cogent dissent was voiced by Allen Roses (Duke University) who argued that cognitive function is depressed to some degree in all DMD patients and mental retardation is simply the consequence of depressing an already low intelligence. An additional complicating factor is the effect of neglect of the disabled child by some families, quite possibly most often those of lower intelligence.

But even assuming the majority view is correct and mental retardation is a distinct phenomenon associated with specific mutations, establishing reliable diagnoses of mental retardation or its absence in all 220 deletion cases available (or at any rate those in which affected individuals are still alive) may demand an effort of organization of a different order from that required for the collaborative effort that led to the original compilation of deletion data on the gene by Kunkel and a total of 72 co-authors⁵.

The clinician's headache

It is no surprise that in a gene of about 2,000 kb the mutation rate and the rate of intragenic recombination, each a serious handicap to genetic counselling and prenatal diagnosis, are high. Two points of particular relevance for clinicians emerged at the meeting, the first (the bad news) arising from the analysis of new mutations. Several participants reported families in which a mother with two apparently normal X chromosomes produced more than one offspring with an identical *DMD* deletion. This means it cannot be assumed that a DMD boy born to parents of normal genotype must himself be a new mutant, and the chance of an

affected sibling thus no higher than in any normal family. There are three possible explanations for the occurrence of identical mutations in the offspring of a mother of normal genotype. The simplest is germline mosaicism: a mutation occurring in a mitosis early in the differentiation of the mother's germ line. A second possibility is a 'pre-mutation' in the mother — a postulate familiar to students of the fragile-X syndrome, in which the mentally retarded phenotype can be silent for a generation or two before reappearing for no apparent reason. More concretely, such a phenomenon could result from a large inversion at the *DMD* locus, which would be undetectable by most probes but could predispose to deletions at chromosome pairing in meiosis (although it is not clear why this should lead to identical deletions). The third possibility (Uta Francke, Yale University) is that in some cases the disease can be autosomally transmitted⁶. Any or all of these mechanisms may account for isolated cases of DMD and each complicates prenatal diagnosis.

The second clinically relevant point to arise at the meeting was the dramatic illustration of the very high proportion of cases that are due to deletions (see ref. 7). Van Ommen's data, which directly test the potential hinted at in three recent papers⁸⁻¹⁰ of pulsed-field gel electrophoresis in prenatal diagnosis, imply that there must be a particularly deletion-prone region at the 3' end of the gene.

Pulsed-field gel electrophoresis (the Leiden group in fact used the field-inversion variant, or FIGE) will separate very large fragments of DNA generated by digestion with rare-cutter enzymes at a resolution of 20–30 kb and can be used for the direct detection of deletions. If, as van Ommen's data suggest, 50 per cent of DMD cases result from deletions detectable by FIGE then half of all prenatal diagnoses should be possible without linkage analysis. Some participants demurred that pulsed-field gel electrophoresis is too sophisticated for routine use, the ideal being an appropriate battery of complementary DNA and flanking probes which would detect small deletions as well as large ones. Others, massively supported by historical precedent, averred that no convenient diagnostic technique was so sophisticated as to escape eventual adoption for clinical use. □

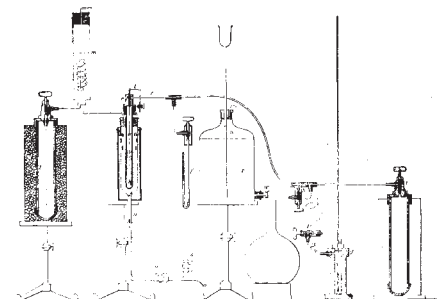
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CONDENSATION OF GASES

AMONG the numerous subjects which have engrossed the attention of the knowledge-seekers of the present century, probably none have surpassed in fascination and in the wealth



of results which have showed persistent effort the question of the possibility of liquefying those gases which for ages had been considered permanent. On the assumption that the molecule of iodine consists of two atoms, which, according to the view now becoming more and more accepted by thinkers on this subject, may themselves consist of aggregations of a still simpler substance — aggregations which, at temperatures obtainable in the laboratory, we have not been able to break up — the classical experiments of Victor Meyer have shown that at a temperature of about 1500°C the molecules are dissociated into single atoms, that is to say, the intensity of the heat-vibrations is so great that the attraction between the two atoms in the molecule is overcome, and they are torn asunder. At still higher temperatures there is a possibility that the atom itself could be resolved into something simpler still.

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Biogeography

How do flightless mammals colonize oceanic islands?

Jared M. Diamond

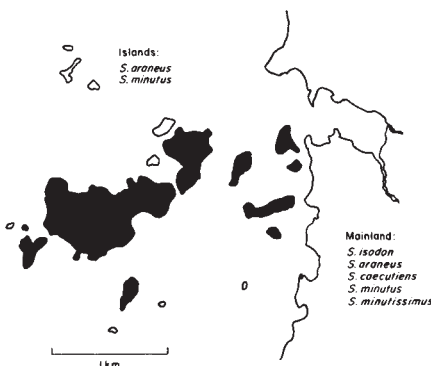
OCEANIC islands support only a subset of an adjacent mainland's species, biased towards species with obvious means of crossing water, such as flight. On the most remote islands like New Zealand and Hawaii the sole native terrestrial mammals are bats, whereas those islands with the richest mammal faunas are ones that had well-documented Pleistocene land bridges to continents, such as Britain and Borneo¹. Thus, it is disconcerting to find that oceanic islands up to the distance of the Galapagos (1,000 km from South America) have any native flightless mammals at all. There has been a long debate over whether such mammals arrived via vanished land bridges undocumented by independent geological evidence, or whether they can somehow, although very rarely, cross water gaps. Several recent studies make the surprising discovery that overwater dispersal of flightless mammals occurs sufficiently often to measure²⁻⁶.

The least implausible mechanisms by which mammals could cross water are by swimming, rafting on floating vegetation or (at high latitudes) rafting on ice floes or crossing frozen straits in the winter. Evidence for water-crossing could include finding a mammal species on an island recently lacking that species, tagging an individual mammal at one site and recovering it on a separate island, and actually observing an individual in transit. All three types of evidence were reported recently by Pokki², who studied voles on coastal islands of the Tvarminne Archipelago, Finland. Of 2,175 voles that he tagged on islands, 98 were recaptured on other islands removed by open-water distances of up to 620 m, and four voles made two inter-island moves. Because most moves were made during the ice-free late summer, dispersal was inferred to have been by swimming, supported by lucky direct observations by two other Finnish scientists.

In Lake Sysmä, Finland, Hanski³ caught 60 shrews on islands that had no breeding populations and that those individuals could thus only have reached by crossing water. One island on which all the shrews were trapped out in 1983 supported a single pregnant female in the spring of 1984, which must have moved to that island after mating elsewhere. Thus, a single colonist could found a population. A shrew can swim for at least 1 h and cover 1 km (see figure, for example). Hanski was not lucky enough to come across

shrews swimming between islands, but other interested 'observers' often do: the stomachs of 2,486 trout yielded 270 shrews!

On Maine coastal islands (northeastern United States), Crowell⁴ recorded newly founded populations of 13 mammal species, which must thus have crossed water in recent decades. The species ranged in size from bear and deer to beaver, raccoon, muskrat, bobcat, fox, rabbit and hare down to voles, mice and



An archipelago in Lake Koitere, East Finland. Dark islands, *Sorex araneus* occurred with *S. minutus* in 1983; light islands, *S. araneus* occurred alone (from ref. 3).

shrews. Deer, moose and otter were actually observed swimming across straits up to 12-km wide.

As a direct test of the role of dispersal across ice, Lomolino⁵ searched for mammal tracks while driving a snowmobile over the frozen St Lawrence River, Canada, in winter. Energetic considerations of winter travel favour large animals, as the ratio of energy reserves to energy costs per kilometre of travel increases with size, whereas problems of heat loss decrease with size. For instance, treadmill experiments show that at near-freezing temperatures a 40-g vole can run 6 km and an 18-g shrew only 1 km. In fact, Lomolino found winter-ice tracks of large mammals to be disproportionately commoner and to go further than tracks of small mammals. In particular, there were six times more vole tracks than shrew tracks, although shrews were more widespread on the mainland.

Those size-related dispersal considerations may account for why voles occur on St Lawrence islands more than 1 km from the shore, whereas shrews only occur on islands less than 700 m from the shore. Size-dependent selection of immigrants may also explain why body size increases with distance for insular populations of

both voles and shrews. Energetic limits on dispersal were further illustrated by the fact that at the end of Lomolino's longest recorded mole track (1 km; 8 times the mean track length), under sub-freezing conditions, was a dead mole.

Many differences between the species composition of island and mainland mammal faunas depend on species differences in over-water dispersal ability. For example, on St Lawrence islands the winter-active mammal species that disperse across ice are over-represented, whereas species that hibernate during the winter ice season are under-represented. Similarly, the increase of swimming endurance with body size favours voles over deer mice on islands of the US Atlantic coast^{4,5}, and favours large rather than small shrew species on Finnish lake islands³.

If dispersal is sufficiently frequent, however, extinction also becomes a determining factor of insular species composition, and Lomolino could interpret species differences in occurrence on islands (incidences) in terms of species differences in ratios of immigration to extinction rates. Thus, perhaps the most surprising result of the recent studies is that populations of not just birds but even of some flightless mammals on islands several kilometres offshore approach immigration/extinction equilibria, as postulated in the MacArthur-Wilson theory of island biogeography²⁻⁵.

For islands tens or hundreds of kilometres offshore, dispersal events will naturally be much rarer. (For example, one successful colonization every few hundred thousand years from Indonesia would have sufficed to build up the mammal fauna of those Philippine islands lying beyond the Palawan land bridge⁶.) Large water gaps imply not only less frequent crossings but also a different mechanism of crossing, namely rafting instead of swimming. Whereas swimming favours large over small colonists, rafting favours small over large colonists. In fact, 19 of the 20 endemic flightless mammal species of Luzon⁶, all those of the Galapagos and all flightless placental species of Australia are small rodents¹. Hence, rafting has left its signature on the native mammal faunas (and perhaps snail faunas, soon to be discussed in a News and Views article by Peter Moore) of distant oceanic islands, just as swimming and ice travel have moulded the faunas of close islands. □

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Lensing of supernova neutrinos?

SIR—The detection of neutrinos^{1,2} from supernova SN1987A has led to an explosion of speculative analyses³⁻⁷. These place a heavy burden of interpretation upon time delays between the individual neutrino events detected on Earth. We would like to point out that the bimodal distribution of the Kamiokande detections could be explained if the neutrino burst from the supernova were split by an intervening gravitational lens. This wildly speculative hypothesis has nevertheless the merit of being testable in the future.

The twelve Kamiokande II neutrino events have arrival times¹ of 0.00, 0.11, 0.30, 0.32, 0.51, 0.69, 1.54, 1.73, 1.92, 9.22, 10.43, 12.43 s and fall into two bunches with nine events around 1 s and three near 10 s. We do not know the intrinsic spread of the neutrino events, but if we assume it to be less than a few seconds then the intrinsic spread of the first bunch is less than the time between the two bunches by a factor of two. Suppose the supernova emitted only a single burst of neutrinos with an intrinsic spread of a few seconds which subsequently encountered the gravitational field of a compact intervening mass, M , en route to Earth. This mass will act as a gravitational lens to produce two (in the case of a point lens) or more (for a distributed lens not dominated by a compact core) neutrino paths. Arrival time differences for the two neutrino signals will, for the point mass lens, be

$$\Delta t \sim \frac{4GM}{c^3} \sim 10 \left(\frac{M}{5 \times 10^5 M_\odot} \right) \text{s}$$

We assume that the ratio of the apparent intensities along the two neutrino paths is 1:3 as indicated by the relative abundance of neutrino events in the two detected bunches. Hence, we see that the observed delay of ~ 10 s between the two bunches of neutrinos could be accounted for by an intervening object with $M \sim 5 \times 10^5 M_\odot$. Assuming the intensity ratio 1:3, with the lensing object at 25 kpc and the supernova at 50 kpc, the image separation angle produced by a point lens is

$$\Delta\theta \sim 0.6'' \left(\frac{M}{5 \times 10^5 M_\odot} \right)^{1/2}$$

Such image-splitting is probably too fine to be resolved at present but future radio, optical, and certainly Space Telescope observations, could check the existence of lensing by searching for the second image of the supernova.

The lens candidate must have a mass $\sim 5 \times 10^5 M_\odot$ within a radius ~ 0.07 pc if the lens lies at a distance of ~ 25 kpc — midway between us and the supernova where the probability of lensing is greatest, although still very small, $\sim 10^{-6}$.

This means that a black hole with a Schwarzschild radius of 5×10^{-8} pc, a compact star cluster, or a compact cluster of 'Jupiters' would be ideal candidates. Both have been proposed for other reasons^{9,10}. If one were to assume that a uniform population of such lens candidates constituted the dark halo of our own Galaxy then the chance¹¹ that one would be found interposed between us and a supernova in the Large Magellanic Cloud is about 10^{-6} .

A more speculative lens candidate is a loop or line of cosmic vacuum string. There has been a recent claim by Cowie and Hu that such an object has been observed producing a unique signature of four pairs of double galaxy images as expected if a string loop passed through four galaxies. A curved cosmic string having mass per unit length μ produces image splitting with angle¹²

$$\Delta\theta \leq \frac{8\pi G\mu}{c^2}$$

A string parameter of $G\mu/c^2 \sim 10^{-7}$ for a string located roughly midway between us and the supernova and approximately perpendicular to the line of sight would also produce the observed delay of ~ 10 s between arrival of the two neutrino signals. The string has to curve, and presumably therefore be part of a loop, for the apparent intensities of the two neutrino signals to be unequal. A string of this sort will produce an image separation of $\sim 0.25''$.

Finally, we should also consider the consequences of including the smaller sample of neutrino data from the IMB detector². IMB saw eight neutrino events at times 0.00, 0.42, 0.65, 1.15, 1.57, 2.69, 5.01 and 5.59 s but with an unknown time origin relative to the Kamiokande events

and a higher detection energy threshold. These two uncertainties make it difficult to compare the two data sets with the information publicly available. If we assume that the time origins are the same then we can draw either of two conclusions concerning the lensing hypothesis. One possibility is that the IMB data provide support for the view that the neutrino arrival times are randomly spread between 0 and 12 s and have no significant sub-structure. Despite the reasonableness of this view it clearly has not been adopted by authors deducing neutrino masses³⁻⁶. The other possibility is to take seriously the bunching of two IMB events near 5 s displayed by the two sets of data in conjunction with each other. In this case the lens hypothesis could imply the existence of three images as predicted for any smooth bounded lens with the intensities of the images in the ratio 1:0.3:0.3.

We thank David Caplin for raising the question of lensing time-delays in connection with supernova SN1987A and also M. J. Rees and T. Padmanabhan for helpful discussions.

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Intracellular pH and cytoplasmic free Ca²⁺

SIR—The title of a recent letter to *Nature*¹ contains a specific conclusion, that "activation of sodium-proton exchange is a prerequisite for Ca²⁺ mobilization in human platelets." Siffert and Akkerman conclude that "an increase in intracellular pH forms an essential step in the cascade of events required to increase cytoplasmic free Ca²⁺ ..." I think that published data from my own laboratory², and from other groups³⁻⁵, argue strongly against this view.

Siffert and Akkerman report that replacement of Na by choline suppresses thrombin-evoked internal discharge of Ca²⁺ and slows and reduces Ca²⁺ influx. Sage and I, however, showed² that replacement of Na by choline had no significant effect on the extent of the rise in intracellular Ca²⁺ concentration, [Ca²⁺]_i, evoked by thrombin or platelet-activating factor in the presence or absence of external Ca²⁺. Also, complete replacement of Na by K had no effect on internal discharge of Ca²⁺. Our data appear to refute

unequivocally the main conclusions of Siffert and Akkerman. I cannot readily explain the discrepancy in the experimental findings; their results might be explained by the use of forskolin and gel-filtration in preparing their platelets, or by the presence of impurities in their sodium substitute. In fact, one result in Siffert and Akkerman's own work seems to show that Na⁺/H⁺ exchange was not an "essential step". Their Fig. 3 shows that at higher concentrations of thrombin there was no significant effect of Na removal on [Ca²⁺]_i rise or on aggregation.

The rightward shift in the thrombin dose-response curves produced by sodium removal, as shown in their Fig. 3, is similar to that reported by Connolly and Limbird³ and attributed to a requirement for Na in the generation of thromboxane A₂. Siffert and Akkerman did not specify the state of cyclooxygenase in their platelets, but presumably they used drug-free donors and their results and their interpretation could be analogous to those of Connolly and Limbird. The finding that ethylisopropyl-amiloride can have similar

effects to those of sodium removal is also consistent with a recent result from Limbird's group⁴ suggesting that this agent can block PLA_2 activation and thus inhibit the release of thromboxane A_2 .

The single record of thrombin-induced elevation of intracellular pH , pH_i , shown by Siffert and Akkerman indicates a delay of some 12 s in the thrombin-evoked elevation of pH_i . So far as one can judge from their paper, the thrombin-evoked $[\text{Ca}^{2+}]_i$ rises seen under similar conditions had no measurable delay; we have shown the delay in thrombin-evoked $[\text{Ca}^{2+}]_i$ rise in human platelets to be less than 0.5 s (ref. 2). These time courses are not consistent with a casual role for elevation of pH_i in Ca^{2+} mobilization.

There is little doubt that thrombin can activate Na^+/H^+ exchange in human platelets. However, the available evidence including that in Siffert's and Akkerman's own figures to me argues against an essential function in Ca^{2+} mobilization. What the physiological role, if any, may be for agonist-evoked elevation of pH_i in human platelets requires further investigation.

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SIFFERT AND AKKERMAN REPLY—In response to Rink's arguments against a function for Na^+/H^+ exchange in Ca^{2+} mobilization in thrombin-stimulated platelets, we wish to emphasize a few aspects of our recent report¹, that are crucial for a proper understanding of it.

First, we demonstrated that Ca^{2+} mobilization depends on Na^+/H^+ exchange — through an increase in cytosolic pH — in the range of about 0.02 to 0.2 Uml^{-1} of thrombin; above that range there is more Na^+/H^+ exchange but on increasing thrombin concentrations the coupling with Ca^{2+} mobilization gradually disappears. Thus, in studies with higher thrombin concentrations² no such coupling can be observed. Support for this conclusion comes from Feinstein *et al.*³, who found that extracellular Na^+ increases the rise in internal calcium ion concentration, $[\text{Ca}^{2+}]_i$, caused by thrombin, and from Sweatt *et al.*⁴, who concluded that Ca^{2+} mobilization is independent of Na^+/H^+ exchange at high thrombin concentrations, whereas at a threshold concentration of thrombin, concomitant alkalization may be required. An appropriate illustration is also found in a report by Zavoico *et al.*⁵, who lowered cytosolic pH with nigericin and found a 35 per cent

reduction in Ca^{2+} mobilization initiated by low doses of thrombin, whereas at high doses of thrombin Ca^{2+} mobilization was independent of cytosolic pH . Probably, weak stimulation of the thrombin receptor results in formation of low amounts of inositol triphosphate (InsP_3 , which triggers Ca^{2+} release from intracellular storage sites. A small increase in pH greatly enhances InsP_3 -induced Ca^{2+} release from isolated intracellular membranes⁶. These considerations are probably more relevant than minor technical differences between the studies by Rink² and our work^{1,7}: both laboratories replace Na^+ by choline and our platelet suspensions show a normal phosphoinositol (PI)-response⁷.

Second, we find no effect of cyclooxygenase inhibitors on the relationship between Na^+/H^+ exchange and Ca^{2+} mobilization. This accords with findings by Hallam and Rink⁸ and Daniel *et al.*⁹, who claimed that cyclooxygenase activity is not required for Ca^{2+} mobilization and InsP_3 formation in platelets that are weakly stimulated, such as by ADP. Connolly and Limbird¹⁰ described that platelet functions induced by ADP, adrenaline and low doses of thrombin (again, not at high thrombin concentrations) depend on external Na^+ and that this response is blocked by cyclooxygenase inhibitors. Thus, they consider Na^+/H^+ exchange and the resulting increase in cytosolic pH major causes for activation of phospholipase A_2 , which would provide the triggers for Ca^{2+} mobilization and secretion, probably by activation of phos-

pholipase C^{11} . Our results are not consistent with this concept and favour a more direct link between Na^+/H^+ exchange and Ca^{2+} mobilization.

Third, we demonstrate that the dependence on Na^+/H^+ exchange is weaker for Ca^{2+} influx from the extracellular medium than for Ca^{2+} mobilization from intracellular storage sites. Thus, especially when time courses are compared between changes in cytosolic pH and changes in cytosolic $[\text{Ca}^{2+}]_i$, the initial mobilization of intracellular Ca^{2+} may reflect more accurately the role of Na^+/H^+ exchange. This aspect of platelet activation requires further investigation.

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How many Chernobyl fatalities?

SIR—In criticizing Robin Russell Jones, Jens Scheer¹ himself makes errors.

There is no way of disproving his proposition that doses below 1 rem produce effects increasing with the square root of the dose, that is in a way suggesting that the early doses reduce the effects of later ones. It does not seem impossible that the cellular system for repair of damaged DNA should improve with use. But a dose of 1 rem will on the average be received by the age of five by children in Britain and most of Europe from the natural background. The square root hypothesis either has no relevance to the effects of later doses received by adult workers in the nuclear industry, most of whom will already have received 5 rem or more before receiving any occupational doses, or implies that the effect of these additional doses is less than would be suggested by the linear hypothesis.

Scheer does not say what he means by a "more correct control group than the traditional 0–9 rad (sic) group". Use of the 1–9 rad group results recorded by Kato *et al.*² gives the largest possible figures for the excess of cancers due to the nuclear bombs on Japan. Kato *et al.* record no statistically significant change at all in the cumulative number of non-cancer deaths (at the 5 per cent level) below 400 rad.

Not only is there no positive evidence for a greater death rate than Russell Jones estimated for the effects of Chernobyl, but his estimates are themselves likely to be too high. There is a great deal of evidence from animal experiments that, by stimulation of the immune system or otherwise, the life expectation of young animals can be increased by quite considerable radiation doses delivered at low dose rates of a few hundred millirem an hour or less³.

The extent to which these results apply to humans is unknown, but neglect of them is likely to have led to a significant exaggeration of the true risks, especially to the young.

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Variations on an enigma

Alan Nunn May

Klaus Fuchs: The Man Who Stole The Atom Bomb. By Norman Moss.
Grafton, London:1987. Pp.216. £12.95.

THIS is a detailed and sympathetic account of Klaus Fuchs's career. The title is misleading, however; no one managed to steal the atom bomb, as the survivors of Hiroshima and Nagasaki can testify. Fuchs passed detailed information about the bomb to the Russians and so helped to end the American monopoly. For that he got 14 years in gaol, not for treason as Norman Moss says, nor for theft, as the title of this book suggests, but for offences against the Official Secrets Act. Hence the age of nuclear deterrence started two years or more earlier than it might have done.

When Western politicians declare that deterrence has kept the peace in Europe for 40 years, they have in mind the deterrence of the Russians. What Fuchs did helped to deter the Americans, which many regard as at least equally important. Thus deterrence has two sides. It is in the effort to see both sides of things that Moss's account of the Fuchs case differs from those by Rebecca West and Alan Moorehead. The author has taken pains to avoid doing a hatchet job, but all the same he has not always succeeded in making Fuchs's motives plausible. Fuchs himself did not give very much help in this enterprise. His confession, and his letters to friends after his arrest, reflect an extreme emotional shock, and are desperate pleas for forgiveness. So one has to guess to some extent what his motives were, and in this some experience of similar events and circumstances may be a help.

Fuchs learned about the Nazi menace the hard way, by being beaten up by Fascist thugs whilst a student in Germany. One sister and his brother escaped, while his father, a distinguished Lutheran pastor, remained in Germany and maintained a determined opposition to the Nazis. He suffered imprisonment, but survived. Klaus and his brother became devoted communists; it must have seemed the only way to put up an effective resistance.

Fuchs escaped to England in 1933 and continued his studies at Bristol under Nevill Mott, producing three classic papers on metal physics. Then he went to Edinburgh to work under Max Born, and again produced three notable papers. All this time he took little part in politics, although he had established contact with German political exiles.

At the start of the war Fuchs at first continued his work in Edinburgh but in

1940 he was interned along with many other refugees and sent to Canada. He was among the first to be released and returned to Edinburgh where he remained until, in 1941, he was recruited for the British atomic project to work under Rudolf Peierls in Birmingham on the separation of uranium isotopes by the diffusion method.

By this time the Germans had turned on

himself, and acted them out in dramatic detail. He also had an inordinate desire to please. Hence his enthusiastic cooperation, first with Russian agents, and eventually with the FBI. By this time the Russians were no longer in acute danger, indeed they were pushing the Germans back. To Fuchs the main danger must now have appeared to be the overwhelming power which the bomb would give to the Americans after the war, power which they might well use to deprive the Russians of all the fruits of their hard-won victory.

In 1944 Fuchs arrived in Los Alamos at the very heart of the bomb project. He was now working on the special problem of the plutonium bomb which was liable to detonate prematurely. The cure was an



From Klaus Fuchs

Place and person — Los Alamos, 1944, where the living conditions were rather basic; and Klaus Fuchs in 1959, on his way to East Germany after finishing his prison sentence.

Russia and were advancing relentlessly. It was a desperate time, especially for many of those in the project who suspected that the Germans were also working on the atomic bomb and might well get there first. It seemed quite possible that in that case they would use it against the Russians, because that was where they were fighting most desperately, and because they regarded the Russians as sub-human, fit only for extermination by any means that came to hand. For Fuchs the temptation to pass on some warning must have been very great. He did just that, and so started on the long story which is the main theme of this book.

At the end of 1943 Fuchs was transferred to the United States, still working on the diffusion process with Peierls. Now there began a series of meetings with Harry Gold, who had two big faults as a contact man. He invented stories about

elaborate system of high-explosive "lenses" driving the plutonium inwards uniformly from all sides — the so-called implosion device. By now the bomb was becoming an immediately practical possibility and urgent discussions started on the question of whether or how it should be used, and whether it should be disclosed to the Russians, and the dangers that would arise if wrong decisions were taken on these points. Fuchs had made his own decision and continued sending information from Los Alamos on the design of the bomb, the rate of production of fissile material and other essential data.

After the war Fuchs was invited to join the British project at Harwell where he was head of the Theoretical Physics Division, and an influential figure. He continued his disclosures but was increasingly uneasy about them. He was becoming disillusioned about Russian policies — this

was the time of the Berlin crisis, the quarrel with Yugoslavia, and a fresh outbreak of purges and trials. He probably also felt that he was no longer revealing decisions and plans made by some remote authority, but plans he had helped to make and with which he agreed. Fuchs seems to have shared fully in the euphoric feeling at the time that nuclear power would save the world from want. For whatever reason, he stopped giving information early in 1949. Then his father was appointed to a professorship in East Germany. Fuchs suddenly felt that his close links with his father would make him vulnerable not only as a security risk, but as an openly apparent one too. So he went to talk to the security man at Harwell — Henry Arnold.

Meanwhile, the Americans had managed to decode some messages sent from New York to Moscow back in 1944. One of these pointed to a leak, and to Fuchs as the source. So Arnold had been already alerted, and for some time had cultivated a close friendship with Fuchs. With Skardon of MI5 he now set about inducing him to confess. Fuchs had experienced the bully-boy methods of the Nazis, but was quite unprepared for the tender-loving-care and friendly-cup-of-tea approach adopted by the wily Skardon. He confessed, and gave a complete list of his disclosures and a description of his contacts. Up to this point the authorities had only known that there had been a leak. Now they found to their horror that nearly every aspect of the project had been disclosed.

After that the trial and conviction were a foregone conclusion. What hurt Fuchs most was that his naturalization was revoked, and it was only then that he told Skardon that he was not really a friend. He earned the full remission of his sentence by good behaviour, was released in 1959, and went to East Germany to rejoin his aged father, and to marry. He was given a senior post in an atomic research institute from which he retired in 1979, covered in honours from a grateful government.

To most Western commentators the period of Fuchs's confession and cooperation with the security services is an interval of normal, right-thinking behaviour in an otherwise deplorable career. To Fuchs things would probably be the reverse. He would regard this period as a brief aberration in a life otherwise devoted to a noble cause. Norman Moss has made a contribution to bridging this gap in perceptions, and has written the best biography of Fuchs so far. □

Alan Nunn May was a member of the British-Canadian atomic project in Montreal from 1943 to 1945. He was convicted of giving the Russians information in 1946 and sentenced to ten years in prison. In 1961 he was appointed Professor of Physics in the University of Ghana, and retired in 1978.

Bishop's vision

M. F. Land

Visual Neuroscience. Edited by J. D. Pettigrew, K. J. Sanderson and W. R. Levick. Cambridge University Press: 1986. Pp. 448. £75, \$125.

Visual Neuroscience arose as a *Festschrift* for Peter Bishop, the unlikely setting being a church hall on Lord Howe Island, in the Tasman Sea. Bishop's career spans the whole post-war period of visual physiology, and our present knowledge of the visual pathways owes as much to the work of his laboratory and those of his students as it does to the pioneer studies of Hubel and Wiesel.

Bishop's own work in vision began in the 1950s with an analysis of the circuitry of the lateral geniculate nucleus, and developed from there into a long series of studies on the nature of binocular vision, and the interactions between the eyes in both geniculate and cortex. In his research papers, Bishop comes across as careful, even cautious, but with a highly inventive methodological streak. He developed much of his own apparatus, and his laboratories were always superbly equipped. Outside his immediate research, Bishop's interests broadened to extend over the whole of vision, and indeed the first paper he admits to, in 1939, was on the nature of consciousness. Arguably, Bishop's real legacy was his students; no less than 25 are now full professors around the world, a statistic that can have few competitors. As Horace Barlow succinctly puts it in his contribution: "... it was not until I collaborated with Bill Levick in the 1960's that I discovered how the professionals — trained

by Peter Bishop — did it".

The book has six sections, beginning with the retina and ending with visual psychology, together with several appreciations of Peter Bishop's career and his full bibliography. It begins with a characteristic chapter by Barlow called "Why Can't the Eye See Better?" — an entertaining re-evaluation of the physical and neural limits to vision. There follow a number of reviews (by Wässle, Levick, Burke, Henry and Kaas) dealing with one of the most important themes of the Bishop school: the X, Y, W classification of retinal ganglion cells and the way this division is maintained through the geniculate and cortex. Kass links the X and Y systems to the "two cortical visual systems" of Ungerleider and Mishkin; the slow, fine-grain X-system leading to recognition in the temporal lobe, and the faster, coarser Y-system subserving orientation in the parietal lobe. There is a short comparative section in which Pettigrew examines binocular vision in cats and owls, and Aitkin contrasts processing in the visual and auditory areas. The final section, "Integrative Aspects", contains a nice pair of papers in which Day explores the phenomenon of illusory contours and Peterhans and her colleagues show how end-stopped cells in the pre-striate cortex can respond to such contours.

The most abiding contribution of Bishop's disciples has been their elucidation of the parallel nature of visual processing, contrasting with the serial hierarchy of the Hubel and Wiesel approach. *Visual Neuroscience* is a considered statement of that position, and is essential reading for all those concerned, at the research level, with the way the brain sees. □

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Diving apparatus — a fur seal fitted with an instrument package to monitor the time, duration and depth of each dive. The picture is reproduced from *Antarctic Science*, edited by D. W. H. Walton and recently published by Cambridge University Press. Price is £25, \$39.50.

The fuzzy side of cytology

Michael Ashburner

Lampbrush Chromosomes. By Harold G. Callan. Springer-Verlag: 1986. Pp.254. DM228.

THE centenaries of two seminal discoveries passed unnoticed a few years ago. The first was that of Balbiani's discovery, in 1881, of the giant polytene chromosomes of flies. The second was the centenary of Flemming's discovery of lampbrush chromosomes, from the oocytes of *Ambystoma*, just one year later. Each of these remarkable structures has contributed enormously to our understanding of chromosome structure and function, and it is particularly timely to pause and take stock.

For the lampbrush chromosomes this is now done in a masterly monograph by "Mick" Callan. Callan is uniquely qualified for the task, for he and his school have been at the forefront of research in this area. But his claim goes further than that. It was work at the Stazione Zoologica in Naples, by Rückert in the 1890s, that established the chromosomal nature of the structures seen by Flemming; and it was in Naples that Callan himself first studied these chromosomes and met his wife, the daughter of Reinhard Dohrn, the director of that remarkable institution, who knew Rückert.

Lampbrush chromosomes are characteristic of the diplotene stage of meiosis in many oocytes. They are also known in the alga *Acetabularia* and in the spermatocytes of *Drosophila*. Their name comes from their characteristic paired loops, which are active in RNA synthesis. For a long time it was thought that these transcripts were of maternal messenger RNAs required for the subsequent development of the zygote. Several discoveries, well reviewed by Callan, make this an oversimplification. Were these RNAs simply to be messengers then the correlation between the sizes of the lampbrush loop transcription units (seen in the famous "Miller spreads") and the species' haploid DNA content would not make much sense. Nor would Gall's discovery of extensive transcription of satellite DNA sequences in the loops. Callan favours the idea that these transcripts are essentially a store of ribonucleotides to be reduced after fertilization and then used as precursors of DNA synthesis — an idea, he points out, that goes back to Brachet in the 1930s. It is interesting that Callan rather underplays the role in amphibia of "true" maternal messengers at a time when these are generating so much interest amongst *Drosophila* biologists.

The transcription of lampbrush loops is only one of the topics covered in this monograph, and other aspects of the chromosomes are likewise reviewed with great attention to the experimental evidence and their historical context. I must admit to being somewhat disappointed that Callan did not allow himself more room for speculation. Every now and again the reader glimpses behind the facts, but too fleetingly. This is a pity, because Callan is well known to be concerned about the deep theoretical issues that studies with lampbrush chromosomes have raised. His paper of 1960, co-written with Lloyd, which appeared in *Philosophical Transactions of The Royal Society*, was the first serious attempt to construct a genetic model of chromosomes that took account of molecular data. His "master-slave" model, published in a fuller form in 1967, was very

influential, despite being based on the conclusion, now known to be incorrect, that the axes of the lampbrush loops move during transcription. Characteristically, Callan dismisses the "master-slave" model in a single line in this book. Nevertheless some of the problems that inspired it, especially the C-value paradox, have not gone away.

Lampbrush Chromosomes will be an essential work of reference for any biologist interested in the structure and function of chromosomes. It will also, I hope, be read as a source of inspiration by molecular biologists, not only as an example of the happiest of marriages between the old and the new, but also for the many puzzles it leaves open for future study. □

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Brought to book

Tony Wright

Water Hyacinth. By Brij Gopal. Elsevier: 1987. Pp.471. \$122.25, Dfl.275.

WATER hyacinth, *Eichhornia crassipes*, is an important weed of freshwaters of the tropics and subtropics. The scientific literature on it is large, growing rapidly and certainly warrants a book-length review. Considering the high price being asked for this book, one might assume that it is outstandingly good. It isn't. For this price, one should at least receive a closely argued and tightly edited book printed to the standard of Elsevier's journals. One doesn't.

Dr Gopal obviously put a lot of effort into collecting information: the book includes a very large bibliography. However referencing and organization of the text are sometimes well below par. In Table 29 (p.223), for example, the moth *Acigona infusella* is presented (correctly) as having been liberated in Australia in 1981 as a biological control agent of water hyacinth and the two references are provided. Two pages later (p.225) it is stated that the moth is expected to be released in Australia in the near future, and two other references are provided. In fact neither of the latter two references say anything of the sort. The first merely reports that host-testing is in progress, while the other gives the date of liberation.

In general the author has failed to bring together separate pieces of related information and comment informatively on them. Even when he does, his attempts can be misleading. The success of biological control of water hyacinth in many countries is well known, yet Dr Gopal states that

The very foundation of biological control (that escape from naturally occurring 'enemies' results in weed growth) is shaken by the case of waterhyacinth as none of the organisms found in Amazonia has proved to be a biocontrol agent effective against waterhyacinth.

In the preface, Dr Gopal claims responsibility for all deficiencies and errors, but he is not being completely fair to himself. It is inevitable that an author will make some mistakes, but it is usual for an editor to find and correct the obvious ones. Clearly, that editing was ineffectual, as is evident right from the start; for example (p.3), "A much more deeper understanding is necessary . . .".

Missing page numbers in cross-referencing — "... is reproduced on p." (p.18) and "see p." (p.43) — are more than just unfortunate in a book costing over two hundred Australian dollars. And I cannot imagine why the usual convention for shortening scientific names was not followed consistently. Thus *Eichhornia crassipes* is shortened to *Eich.* *crassipes* as well as to *E. crassipes*, *Pontederia* to *Pont.* (p.18) and *Ancylloscelis* to *An.* (p.103). So much for the editing. The standard of printing too is poor. Crooked lines and broken letters abound, and word spacing and alignment are particularly erratic where italics (or rather the type used instead of italics) are used.

There are many more errors and imperfections, and most pages have one or the other. This is a great shame, because they mar a volume which could have been very good. Without a commitment to improving the quality of its books, Elsevier may find few authors willing to help complete the potentially worthwhile series, "Aquatic Plant Studies", in which this is the first title to appear. □

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The puzzle of the preface

Peter Harman

Mach I, Mach II, Einstein und die Relativitätstheorie: Eine Fälschung und ihre Folgen.* By Gereon Wolters. *Walter de Gruyter*: 1987. Pp.474. DM 188.

"It is justified to consider Mach as the precursor of the general theory of relativity." Writing in 1930, Einstein expressed his estimate of the importance of the work of the Austrian physicist and philosopher Ernst Mach (1838–1916) in these generous terms. Einstein had read Mach's *Science of Mechanics* (1883) as a student, and had encountered Mach's critique of the newtonian concepts of absolute space and motion. The two men had corresponded and met; and Mach had publicly expressed approval of Einstein's work in the second (1909) edition of his *Conservation of Energy*.

It has therefore long seemed an enigma that Mach should have attacked relativity theory in the preface, dated July 1913, to his *Principles of Physical Optics*, published posthumously in 1921. There it is

* *Mach I, Mach II, Einstein and the Theory of Relativity: A Forgery and its Consequences.*

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

The young Einstein (left) and Ernst Mach — was it Mach or his son who wrote: "I reject the present-day relativity theory, which I find to be growing more and more dogmatical"?

stated in trenchant terms that Mach wished to cancel his expressions of approval of the theory of relativity: "I reject the present-day relativity theory, which I find to be growing more and more dogmatical". Einstein himself was undoubtedly disappointed by Mach's unexpected criticism, which he attributed to old age.

In this new contribution to resolving the mystery, Gereon Wolters argues that Ernst Mach (whom he denotes Mach I) was not the author of the preface; he maintains that it is a forgery, the forger being Mach's son Ludwig (Mach II). Ludwig was not therefore merely Ernst Mach's heir and literary executor, but (according to Wolters) took it upon himself to complete his father's work.

There has of course been considerable debate on the reasons for Mach's change of heart. It has been suggested that Mach was influenced by the philosopher Hugo Dingler, who subsequently certainly adopted a strongly antagonistic stance towards the theory of relativity; and that Mach had come to recognize Einstein's divergence from Machian philosophy, which emphasized the primacy of sensations in determining the structure of reality. In their efforts to resolve the puzzle, interpreters of Mach's philosophy have stressed the divergence between the views of Mach and Einstein; Wolters emphasizes their consonance.

Mach had a richly varied career as a scientist, embracing physiology and psychology as well as physics. His 1887 paper on supersonics subsequently led to the introduction of the term "Mach number". It is however his substantial work on the history and philosophy of physics, and on epistemology, which is relevant here. The aim of Mach's critical and historical studies of physics was to depict the factors which lie behind the adoption of scientific concepts. He emphasized that the concepts of classical physics were historically

contingent rather than logically necessary, being acquired in a complex process of historical development. He criticized Newton's doctrines of absolute space, time and motion on the ground that they could not be established by experimental observations. He concluded that the motions of bodies should be considered relative to all observable matter in the Universe, inertial mass being specified by a dynamical functional relationship between a body and the rest of the Universe; this argument was later generalized by Einstein as "Mach's principle". Contemporaries recognized Mach as a progenitor of relativistic physics.

Wolters's argument for the forgery of the preface to the 1921 *Optics* by Ludwig Mach is by no means conclusive. It is based on a study of Ludwig Mach's career and character, and his relationship to Dingler. Wolters is however justified in claiming strong probability for this thesis. It has the merit of simplicity, resolving an awkward textual puzzle. Wolters aims to clarify the confusion in the literature over Mach's attitude to relativity theory and his relation to Einstein. These are the consequences (*Folgen*) referred to by Wolters, and he does give rather too much attention to polemics with previous interpreters of these matters.

Einstein's philosophy undoubtedly moved away from Mach's positivism, and his relation to Mach will no doubt continue to occupy commentators. Nevertheless, Wolters's monograph is a substantive study which, at the very least, provides a plausible interpretation of the origins of the preface to Mach's posthumous *Optics*, and offers a solution to the puzzle of Mach's attitude to relativity theory. □

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Rank injustice in academic research

Raphael Gillett and Marilyn Aitkenhead

The assessment of British university departments carried out last year by the University Grants Committee was badly flawed. Coupled with increased selectivity in funding, the consequences could be serious.

IN MAY 1986 the University Grants Committee (UGC) announced the results of its comparative evaluation of the research performance of university departments in Britain. This article addresses three main issues arising from the UGC exercise. The first is the decision by the UGC to withhold information about the criteria used, thereby precluding informed public debate about their suitability. The second is the soundness of the design and the validity of the measures, and hence the accuracy of the UGC ratings themselves; in one discipline after another evidence is accumulating which reveals serious anomalies in the ratings¹⁻³. The third issue is to do with the policy implications of the results, particularly regarding the distribution of funds between and within universities.

The fact that the UGC chose not to reveal or discuss the criteria it used is cause for concern. Secrecy is the enemy of good science, the essence of which is that assumptions and procedures are open to public scrutiny so that findings may be tested by independent replication and by detailed critical evaluation. Moreover, by refusing to discuss its assessment procedures the UGC was unable to benefit from the considerable expertise in the field of assessment and evaluation to be found in the academic community at large.

It is not surprising, therefore, that fundamental methodological errors have been identified in the design of the UGC exercise¹⁻³. These include: (i) a failure to undertake a comprehensive analysis of perhaps the most valid and direct indicator of research performance, namely, actual research output; (ii) sampling artifacts in the design which contributed to a pronounced bias in favour of larger departments; and (iii) the use of measures of suspect validity (for example, research income), which are known to be strongly confounded with factors unrelated to research performance.

The failure of the UGC to undertake a full and thorough analysis of actual research output is astonishing, given that a department's publication record provides arguably the most direct and valid indication of its research achievement. The seriousness of this omission is only now being appreciated in the light of recent, independent studies in anatomy and physiology¹, German² and psychology³, all highlighting major mismatches between

the UGC ratings and the actual research output of departments.

In the last of these studies, one of us (RG) conducted an analysis of the publications (books and articles in learned journals) of almost every psychology department in Britain for the year 1984-1985. Actual research performance was compared with the UGC's ratings. It was found that the UGC ratings bear no relation to the actual research output of departments in that year. The ratings are

"Particularly disturbing is the finding that a number of departments rated by the UGC as 'below average' actually produced more and better research on average than several departments rated as 'outstanding'."

unrelated to either quantity or quality of research as measured by internationally recognized, objective indices. The indices used were average number of publications and average citation impact, both averages being based on the total number of lecturers and full-time research workers in a department. The UGC ratings correlate $r = 0.01$ with average number of publications, and $r = 0.03$ with average citation impact. In terms of these indices, the UGC ratings have approximately zero validity. Particularly disturbing is the finding that a number of departments rated by the UGC as "below average" actually produced more and better research on average than several departments rated as "outstanding".

There is evidence that the UGC assessment was biased in favour of larger psychology departments. Of all the variables examined in the study, departmental size is the one with which the UGC ratings correlate most strongly. A correlation of $r = 0.48$ was found. This finding might be of less concern if departmental size were itself positively correlated with research performance. However, it is not. The correlation of departmental size with average number of publications is $r = -0.05$, and with average citation impact is $r = -0.09$. The latter results are of interest in their own right, because they cast doubt on the currently fashionable view that "big departments are best".

The anomalies in the UGC evaluation of psychology departments cannot be

explained away by differences in material resources between departments (such as amount of recurrent grant and equipment grant) — at least two smaller, less well-funded departments succeeded in producing research of greater average quantity and quality than a wealthier department around twice their size. Curiously, the smaller departments were rated by the UGC as "below average", while the larger department was rated as "outstanding".

In other disciplines a similar bias in favour of larger departments may also be present — there is a sampling artifact in the design of the UGC evaluation which can lead to the overestimation of their performance. Departments were asked by the UGC to select five recent publications which their respective universities would regard as "typical of the best" of their research⁴. These five publications formed one of the bases on which a department's research achievement was judged.

Now, if a large and a small sample are drawn from the *same* population, the mean of the k highest scores from the large sample is, in general, likely to be greater than the mean of the k highest scores from the small sample. The larger the difference in sample size the greater the margin by which the mean of the k highest scores of the large sample is likely to exceed that of the small sample. To gain an idea of the magnitude of this bias, suppose that two departments of 6 and 29 members of staff (the sizes of the smallest and largest psychology departments) each produced one publication per person. Suppose also that the quality of research in both departments is described by the same normal distribution. The expected mean quality of the five best publications is simply the expected value of the mean of the top five order statistics of the normal distribution. From Table 2.4 in Neave⁵, the expected mean of the top five standard normal scores for a sample of 6 is 0.25, and the corresponding mean for a sample of 29 is 1.43. In other words, the magnitude of the bias in favour of the larger department can exceed one full standard deviation.

Sheppard *et al.* have drawn attention² to a second sampling artifact which may have compounded the errors produced by the first one. In certain subject areas, some departments "had three sides of A4 to themselves and could prove their worth by citing five publications", while others "had to share the three sides and five

publications" with other departments.

As far as can be ascertained, none of the measures of research achievement used by the UGC has ever been validated in a well-controlled, independent study. All are indirect indicators and are confounded with factors unrelated to research performance. Indeed, as has been demonstrated in the case of psychology, the aggregate of these measures, in the form of the UGC ratings, bears virtually no relation to actual research output.

Research income is believed to be one of the main performance indicators employed by the UGC. Now, the number of grants held by a department does correlate with the total number of publications it produces. But this cannot be taken as an indication of the validity of the measure. Grants are specifically given to stimulate research activity. With the aid of a research assistant obtained through a grant, an academic can produce perhaps twice as much output as before. Therefore, even if research grants were handed out randomly, there would be a positive correlation between research income and number of publications produced.

There are a number of difficulties in using research income as an indicator of research strength. First, it discriminates against departments whose research effort is concentrated in those fields, however central to the discipline, where grants are in relatively short supply. Second, credit is given simply for receiving a grant, irrespective of the quantity and quality of research it generates. Third, additional publications produced with the help of a grant may artificially enhance a department's average publication rate. This occurs if the traditional (but inappropriate) method of calculating a department's average publication rate is employed, whereby total number of publications is divided by number of lecturing staff, omitting to include research staff or to take account of material resources.

False appeal

The false appeal of this type of index has been the subject of research in its own right. Consider the circumstances in which grants are awarded by agencies such as the Science and Engineering, Economic and Social, and Medical Research Councils. First, there is a potentially large number of strong, well-qualified applicants, i.e. the base rate is high. Second, there are only a few grants to go round, i.e. the selection ratio is low. Third, a grant confers an advantage on the recipient, i.e. there is a treatment effect which enables the grant holder to produce more and/or better research than those who do not have this form of assistance. It is through a failure to appreciate the combined influence of these three factors, that research income is frequently perceived to have a much greater ability to predict research

performance than it in fact possesses. Such misplaced confidence in highly fallible decision-making strategies has been termed the "illusion of validity"⁶. The mechanisms governing the persistence of the illusion of validity under the circumstances just outlined have been examined in detail by Einhorn and Hogarth in their analysis of overconfidence in judgement⁷.

Turning to some of the policy implications of the UGC exercise, it is clear that already the UGC ratings are being used as a basis for the allocation of funds both between and within universities. This is worrying, not only because of the serious anomalies in the ratings, but also because of the lack of clarity in the conceptual basis for the UGC's policy recommendations. Assuming that strengths and weaknesses can be identified, how should resources be distributed?

The UGC favours increased selectivity, that is giving more resources to stronger departments and fewer to weaker ones. However, this could be a poor strategy for a number of reasons. On the one hand, the law of diminishing returns might apply, causing the gain in productivity by the strong to be outweighed by the loss in productivity by the weak. On the other hand, if research performance is linearly related to the amount of funds received, then giving more to the strong and less to the weak will produce much greater variation in quality across departments than currently exists. The poorer departments may well be consigned to a downward spiral, with possibly severe consequences for research output in Britain, for staff morale, for the brain drain, and for educational standards in those universities where the vital link between teaching and research has been broken.

A related recommendation of the UGC is that small departments should be avoided, and already this recommendation is being implemented. Presumably, this policy rests on the assumption that there is a positive relationship between departmental size and research performance. However, as noted earlier, recent evidence³ casts doubt on this assumption.

Clearly, the policy of increased selectivity in funding needs to be thought through more carefully. Its underlying assumptions should be rigorously scrutinized and its possible consequences actively explored. Alternative strategies should be considered, such as the more equitable distribution of resources, because they may conceivably yield greater net returns for both research and education.

Given that increased selectivity has potentially deleterious consequences, it might be expected that the UGC would have provided a reasoned argument containing convincing justifications for such a policy. It has not. The UGC states⁸ merely: "We propose to adopt a more selective approach in the allocation of

research support among universities in order to ensure that resources for research are used to the best advantage" (p.5). The UGC does not say precisely *how* increased selectivity will lead to the best use of resources. This course of action is simply assumed to be optimal without any analysis of its merits or any exploration of alternative strategies.

Moreover, it can be argued that increased selectivity is actually incompatible with some of the UGC's stated objectives for the university system. For example, the UGC notes⁸ that: "It is of the essence of basic scientific research that its outcomes are unpredictable and that it frequently flowers in unexpected places. Its funding must therefore provide the necessary degree of flexibility" (p.18). However, by its very nature, increased selectivity will make innovative research all but impossible in those departments that are starved of resources.

Teaching

Teaching as well as research will almost certainly suffer as a result of the UGC's policy. As the UGC itself states⁸: "... the close association of teaching and research gives a special strength and vitality to the universities. Able young people receive a unique stimulus from being taught by those engaged in extending the frontiers of their discipline" (p.16). By weakening this close association, increased selectivity will threaten the quality of teaching in universities.

The anomalies in the UGC assessment of research performance, when combined with increased selectivity, may lead to the contraction or closure of departments with an excellent research record. Because of their profound and lasting implications for universities, it would seem desirable that future evaluations of research performance should take place in the context of a public and broadly based debate. To ensure an acceptable level of validity, the measures to be employed need to be discussed, and their underlying assumptions need to be articulated and, where possible, tested. □

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Finger protein of novel structure encoded by *hunchback*, a second member of the gap class of *Drosophila* segmentation genes

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The DNA sequence of hunchback, a Drosophila segmentation gene of the gap class, reveals that like Krüppel, the first gap gene analysed, it encodes a protein with finger motifs typical of a class of DNA-binding proteins. Unlike Krüppel, hunchback encodes a maternal transcript, which becomes graded along the longitudinal axis of the embryo. This, together with other features of its pattern of expression in the zygote, suggests specialization among the gap genes.

EMBRYOLOGICAL^{1,2} and genetic³⁻⁶ experiments provide compelling evidence that the segment pattern of the insect body is specified by a dynamic system of spatial cues generated at the blastoderm stage of embryogenesis⁷⁻⁹. Each of the genes providing these spatial cues is defined by characteristic deletions of segments in embryos in which the gene is mutated. Genetic analysis has allowed three classes of zygotic segmentation genes, which relate to three different levels of spatial organization during segmentation, to be distinguished¹⁰. Gap genes act on the entire egg as a developmental unit, pair-rule genes act on double segmental units and segment polarity genes on the single segment level¹⁰. Analysis of the regulatory interactions among these¹¹⁻¹⁵ supports the theoretical notion¹⁶ of a hierarchical determination of the segmentation process, in which the gap genes constitute a distinct class of genes linking the maternal information stored in the egg with the spatially coordinated zygotic expression of the members of the other classes of segmentation genes.

The gap class of segmentation genes includes *hunchback* (*hb*), *Krüppel* (*Kr*), and *knirps* (*kni*) (ref. 10). Mutations at the gap loci cause deletions of adjacent segments in the anterior (*hb*), middle (*Kr*) and posterior (*kni*) region of the embryo¹⁰. Amorphic *hb* embryos have gnathal and thoracic segments deleted. In addition they show fused abdominal segments 7 and 8, and an abnormal telson¹⁷. Weaker alleles show less severe deletions in the segment pattern and can be ordered into a phenotypic series¹⁷. In contrast to *Kr* and *kni*, *hb* shows maternal

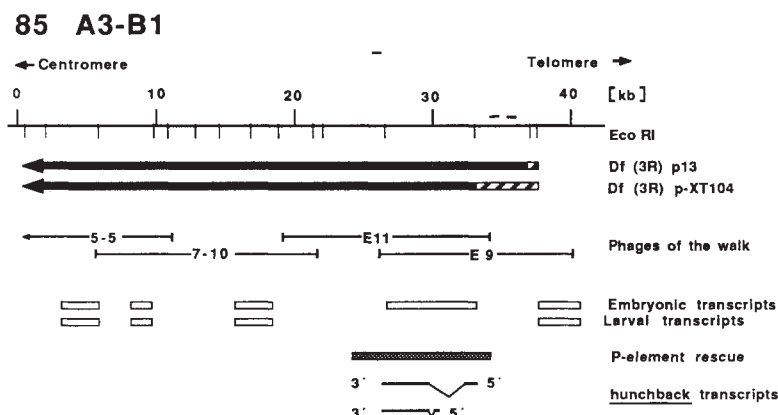
as well as zygotic activity. Absence of the maternal *hb*⁺ activity results in a more severe deletion phenotype which can be paternally rescued¹⁷. This indicates that maternal information contributes to the zygotic phenotype, but by itself is not decisive for normal development. Furthermore, several *hb* alleles show homoeotic transformations of head into abdominal segments¹⁷. These transformations imply that *hb* might be more directly involved in the regulation of homoeotic genes than the other gap genes. The *hb* gene product might be required to suppress directly or indirectly homoeotic gene activities in the head and possibly also in the thoracic region of the embryo. The interaction of *hb* with genes of the bithorax complex becomes directly apparent in the *hb* allele *Rg(pbx)* which exerts a dominant *trans*-regulatory function on *postbithorax* (refs 18, 19), as well as by the observation of ectopic expression of the *Ubx*⁺ gene in *hb* mutant embryos²⁰.

Here we report the isolation and the structural features of the *hb* gene as well as its temporal and spatial patterns of expression. We compare them to *Kr*, the gap gene that has recently been examined using molecular techniques²¹⁻²³.

Cloning and identification of *hb*

The *hb* locus maps to the region 85A3-B1 of the right arm of chromosome three and distal to *pink* (ref. 17), an eye-colour mutant²⁴. Cloning of *hb* was begun by extending a chromosomal walk within the *pink* region. The distal breakpoints of two deficiencies (see Fig. 1) uncovering *pink* and *hb* were

Fig. 1 Chromosomal walk in the *hb* region 85A3-B1 of the right arm of chromosome three. Upper line, scale in kilobases (kb) and location of the *Eco*RI restriction sites. Arrows below, regions which are deleted in two deficiencies which uncover *pink* and *hb* (refs 17 and 18). The breakpoints were only approximately mapped and the uncertainty region is indicated by shading. Only four selected phages from the walk are shown below this, phages E11 and E9 were isolated from a Canton-S genomic library in EMBL4 vectors²¹. Transcripts in the region were localized by hybridizing oligo(dT) primed radioactive cDNA made from 2-5 h and larval poly(A)⁺ RNA to restriction digests of phage DNA. Boxes, approximate location of transcripts. We cannot exclude the possibility that there are additional rare, or poly(A)-free transcripts in the region. The fragment providing the P-element-mediated rescue is bordered to the left by a genomic *Bam*HI site and to the right by the end of the insert in phage E11, which provides the second *Bam*HI site. The location of the *hb* transcripts, derived from further analysis, is shown below this. Standard methods were used for DNA and RNA work⁴⁵.



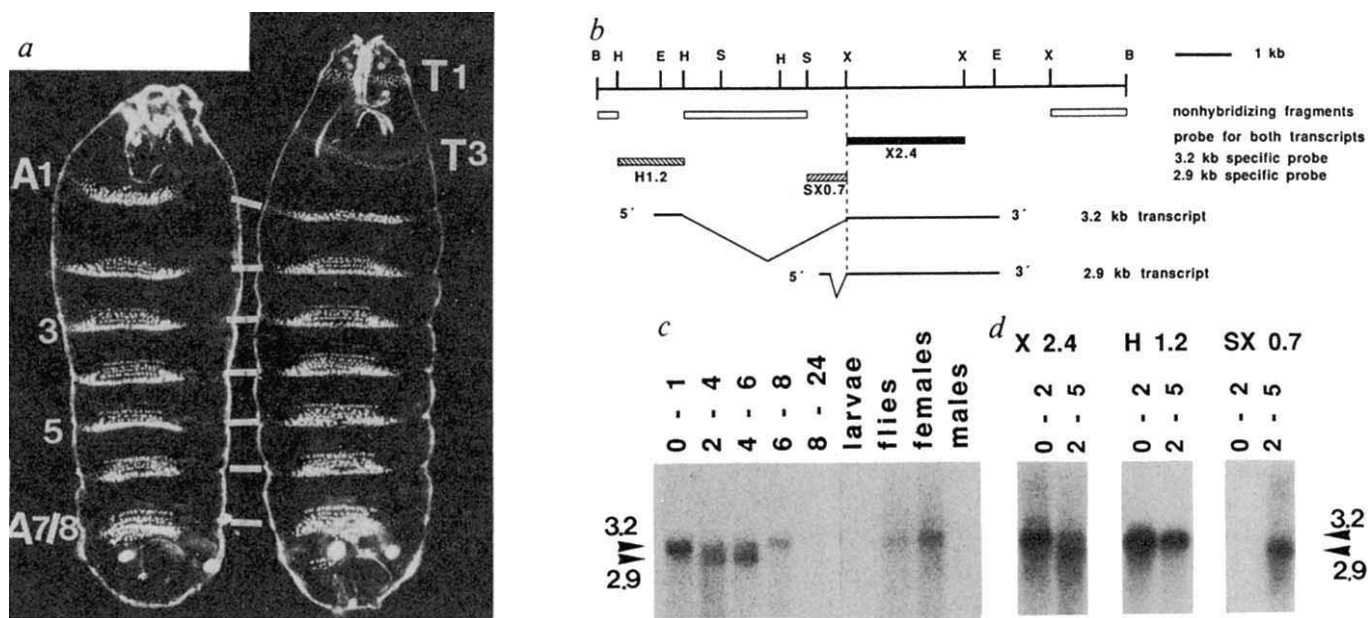


Fig. 2 P-element-mediated rescue and Northern blot analysis. *a*, Cuticle preparations of homozygous *hb* (left) and rescued larvae (right) are shown. We used the amorphic point mutation *hb*^{14F} (ref. 17) for these experiments. In the rescued larvae the head skeleton and the thoracic segments T1 and T3 are always formed, whereas T2 is partially present in some cases. T1 can be recognized by the thick denticles and the patch of hairs posterior to the denticle band on the ventral side. In some cases, the abdominal segments A7 and A8 did not show a complete fusion (as shown), but this is also occasionally observed in strong *hb*⁻ embryos. *b*, Restriction map of the rescue-providing DNA region and relative location of the two transcripts. Boxes, fragments used in Northern blot hybridizations. B, *Bam*HI; H, *Hind*III; E, *Eco*RI; S, *Sal*I and X, *Xba*I. The rightward *Eco*RI-*Xba*I fragment (not shown as a box) does hybridize weakly with both transcripts, indicating that the poly(A) addition site must be a short distance beyond the *Eco*RI site (compare Fig. 5). *c*, Developmental Northern blot probed with the 2.4-kb *Xba*I fragment common to both transcripts. Numbers above lanes, hours of development after egg collection at 25 °C. *d*, Northern blots probed with transcript-specific probes, as indicated above the blots. Longer exposure of the blots reveals additional minor transcripts, which could arise from differential use of the polyadenylation signals at the 3' end of the gene (see Fig. 5). Size markers in *c* and *d* in kilobases.

Methods. Cuticle preparations were as described¹⁷. Northern blots were prepared from glyoxylated RNA samples run on 1% agarose gels, using 5 µg poly(A)⁺ RNA per lane. RNA samples were prepared from embryos and adults of the *Drosophila* Oregon P2 wild-type stock.

molecularly mapped in the cloned DNA. The cloned region between *pink* and the breakpoints of the deficiencies should therefore have contained essential sequences of the *hb* gene.

Transient expression of injected genes encoded by cloned DNA can lead to partial rescue of the phenotype of mutant embryos²¹. To identify the region possessing *hb* gene function, we injected cloned phage DNA into an anterior position of syncytial blastoderm stage embryos from *hb*/TM3 parents (TM3 is a balancer chromosome, ref. 24). DNA from two phages (E11 and E9 in Fig. 1) rescued a small proportion of the injected *hb* embryos (3 out of 41 with E11 DNA and 2 out of 35 with E9 DNA). In each case, the phenotypic rescue was rather weak, showing the development of no more than one additional ventral denticle band. This initial experiment allowed tentative assignment of *hb*⁺ function to the region of overlap between the two phages (Fig. 1).

For unambiguous identification of the *hb* gene, we used P-element-mediated germline transformation to attempt a genetic rescue²⁵. A 10-kilobase (kb) genomic DNA fragment which extends the overlap of clone E11 and E9 slightly (Fig. 1) was subcloned in the Carnegie 20 vector which contains the *rosy*(*ry*) wild-type gene as a genetic marker²⁶. DNA from this 'C20-*hb*' construct was injected into *hb*,*ry*/TM3,*ry* embryos. From their progeny, three independent *ry*⁺ transformant stocks were established. Homozygous *hb* embryos carrying one copy of the C20-*hb* DNA develop a normal head and normal pro- and metathoracic segments, but the mesothorax and the posterior *hb* domain were still abnormal (Fig. 2*a*). Because all three transformant stocks showed the same phenotype, this is unlikely to be the result of a position effect. The phenotype of the transformants is novel and has not been observed in weak *hb*

alleles, which always show a link between the strength of the defect in the anterior and posterior domains¹⁷. To show genetically that this novel phenotype results from the C20-*hb* construct, we introduced the chromosome carrying the C20-*hb* DNA into a *hb* stock marked with the homoeotic mutation *Ubx* (ref. 17). This allows homozygous *hb* embryos to be distinguished from their siblings on the basis of the *Ubx* phenotype, namely, transformation of the first abdominal segment into a thoracic one. All embryos of the rescued phenotype described above showed this transformation. This proves that the novel phenotype is observed only in *hb* mutant embryos and that it results from partial rescue by the C20-*hb* DNA. The transformed embryos are not viable, indicating that one copy of the 10-kb DNA fragment used for transformation is insufficient to restore full *hb* wild-type function. The addition of a second copy of C20-*hb* DNA to mutant embryos had no significant effect on either the phenotype or on viability. Thus essential regulatory elements upstream and/or downstream of the 10-kb fragment are also required for both normal segmentation and survival of the embryos.

Two related transcripts from *hb*

Our walk distal to *pink* encodes at least five different transcripts. One of them maps to the DNA fragment which provides the germ-line-mediated rescue (Fig. 1) and is specifically expressed in embryonic RNA, as expected for *hb* from genetic analysis^{17,18}. Therefore, this transcript seemed likely to contain the *hb* coding sequences. Northern blot analysis with the corresponding DNA fragment revealed two main transcripts of 3.2 and 2.9 kb, which are developmentally regulated (Fig. 2*c*). The 3.2-kb transcript is present in the maternal RNA and continues to be present for

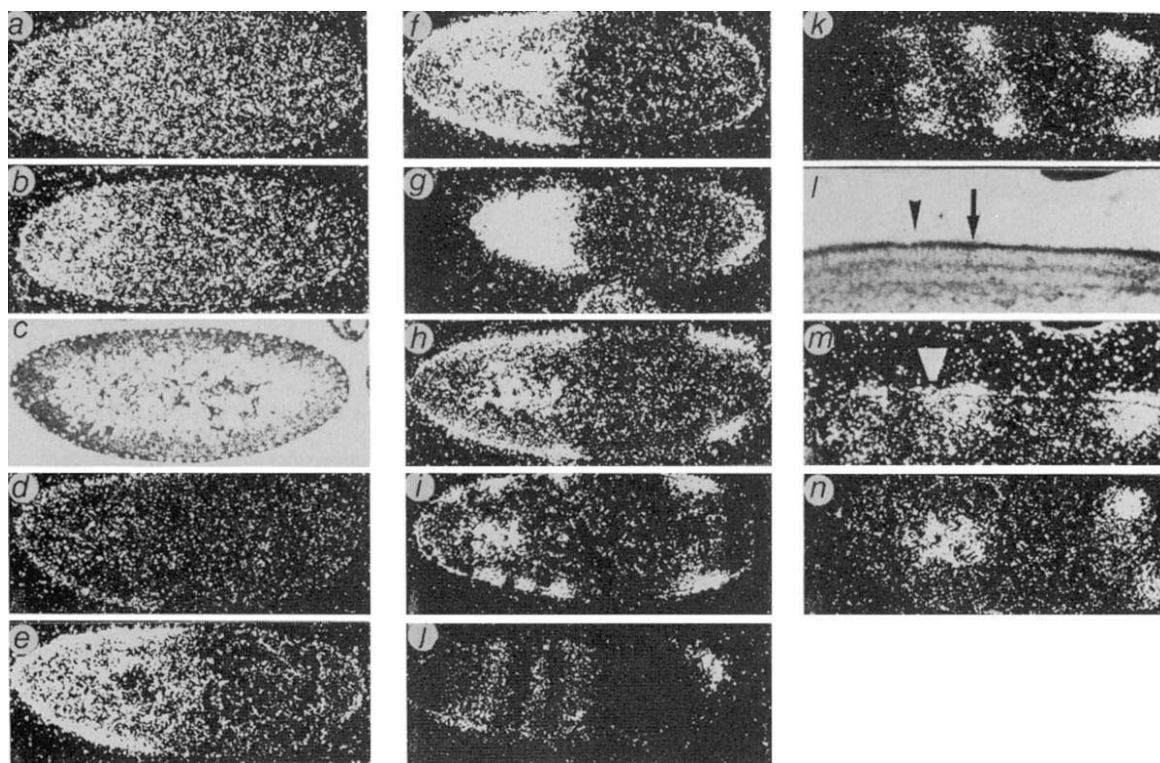


Fig. 3 Spatial patterns of *hb* transcripts during early *Drosophila* embryogenesis. Tissue sections of wild-type embryos during early cleavage up until the beginning of gastrulation were hybridized *in situ* with a 1.4-kb cDNA probe from the 3' end of the RNA and common to both transcripts. Darkfield photomicrographs showing the accumulation of *hb* transcripts at different stages of development; the stages refer to the number of nuclear divisions³⁰. Orientation of sections is anterior left and dorsal up. *a*, Sagittal section of an embryo before stage 8 showing an almost homogeneous distribution of transcripts. *b*, Sagittal section of a stage-9 embryo showing higher levels of *hb* transcripts in the anterior region of the embryo. *c*, Bright-field photomicrograph of a parasagittal section of a stage-11 embryo and *d*, dark-field view of the corresponding section showing first zygotic *hb* transcripts covering the nuclei in the anterior region of the embryo. *e*, Sagittal, *f*, horizontal and *g*, tangential section of stage 13 embryos showing the additional accumulation of *hb* transcripts in the posterior domain. *h*, Sagittal section of an early stage-14 embryo. Note the low levels of transcripts at the anterior and posterior poles. *i*, Horizontal section of a mid-stage-14 embryo. Note the absence of transcripts in the posterior pole region and the beginning of a striped pattern in the anterior domain. *j*, Tangential lateral section of an embryo at the same stage. The anterior domain is split into a region of three stripes. For the sections in *i* and *j* we chose embryos that showed this pattern particularly clearly. Because we found only very few of them, we assume that the three-striped pattern is either transient or not fully penetrant. *k*, Tangential lateral section of a late stage-14 embryo showing two stripes of *hb* transcripts in the anterior domain. The tilting of the stripes towards the anterior is an artefact of this particular section. *l*, Bright-field photomicrograph of a sagittal section of a late stage-14 embryo. The photomicrograph is enlarged and shows the dorsal portion of the embryo in the region between 10% and 90% EL. Arrowhead, cephalic furrow, which serves as a landmark in this embryo. Arrow, end of the domain of hybridization, which is about 10–12 cells posterior to the cephalic furrow. *m*, Dark-field photomicrograph of the section shown in *l*; arrowhead, cephalic furrow. *n*, Sagittal section of an embryo at the beginning of gastrulation. Note that *hb* transcripts continue to be present in the yolk cells of the anterior domain but not at the periphery, and that the posterior domain has not changed between stage 14 and this stage.

Methods. Preparation of tissue sections and the hybridization conditions were exactly as already described²² using ³⁵S-labelled DNA fragments. Serial sections were used as control for orientation of the embryos, for precise staging and for determination of the spatial *hb* domains involving the *Kr* and *fushi tarazu* probes as molecular markers on adjacent sections (compare ref. 15).

the first 8 h of embryonic development. The 2.9-kb transcript is detected only between the second and sixth hours of embryonic development. Both transcripts are absent in larval RNA and in RNA from adult males. The 3.2-kb transcript is detected again in adult females, as is consistent with its presumptive maternal origin. Detailed mapping of the two transcripts (using strand-specific probes in combination with various genomic subfragments; Fig. 2*b*) revealed that both RNAs are transcribed from the same strand and that they differ only in their 5' regions. The 3.2-kb transcript contains an additional 500-basepair (bp) exon which is separated by a 3.2-kb intron from the rest of the gene. The exact splice sites for this intron were determined by sequencing a complementary DNA fragment from this region and comparing the sequence with the corresponding genomic DNA (Fig. 5). The 5' end of the 2.9-kb transcript lies in this intron because this transcript hybridizes in Northern blots specifically with a probe from the 3' end of the intron (Fig. 2). The leader of this transcript is also separated by a small intron from the rest of

the gene. The acceptor splice is the same for both transcripts as inferred from S₁ mapping (see Fig. 5 for details).

Spatial pattern of *hb* expression

Embryos collected between 1 and 4 h after egg laying were probed for *hb* and *Kr* transcripts in alternating serial sections, the pattern of *Kr* transcripts²² providing an internal control for assaying the distribution of *hb* transcripts. After egg deposition and before the 8th nuclear division, *hb* transcripts are evenly distributed within the embryo. After pole cell formation and up to embryonic stage 11 (ref. 27), when nuclei continue to divide at the periphery of the egg, transcripts appear to be distributed in a graded fashion along the longitudinal axis of the embryo (Fig. 3). To follow the fate of the maternal *hb* transcripts in the absence of zygotic *hb* gene expression, we probed embryos derived from *hb/TM3* parents. In a quarter of these, the presumed *hb* homozygous fraction, we observed a continuous decrease in the quantity of maternal *hb* transcripts, which fell

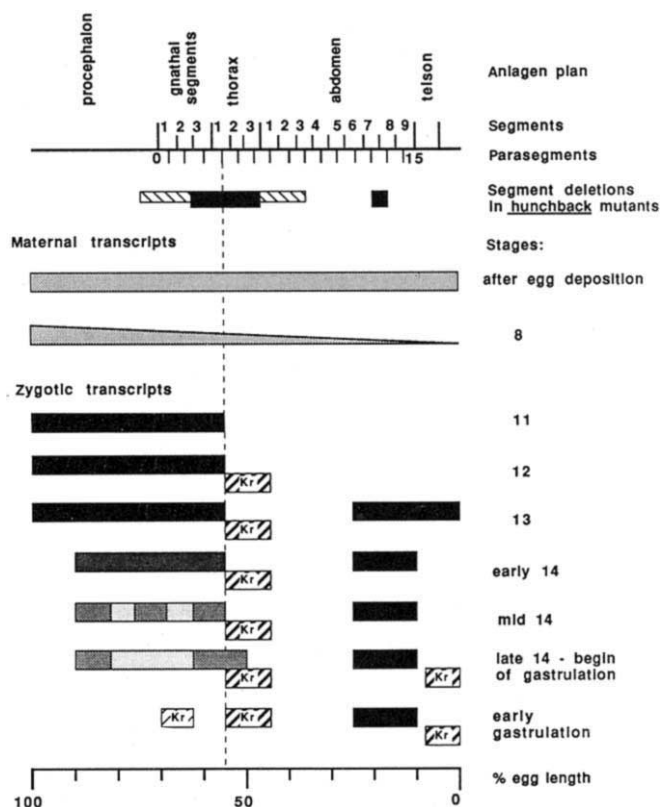


Fig. 4 Summary diagram of the *in situ* hybridization results including a comparison with *Kr*. The observed distribution of grains along the embryo at the different stages is symbolized by the shaded bars. The degree of shading is roughly proportional to the intensity of the observed signals. The spatial extent of the hybridization signal was measured in %EL and related to the Anlagen plan by double hybridizations with the *fushi tarazu* probe¹⁵. Similarly, the exact relative location of the *Kr* expression domain was determined by double hybridizations and/or hybridizations to alternate sections. The *Kr* expression domains are included in the figure (striped and marked '*Kr*'). Black bars below the Anlagen plan show the extent of segment deletions in *hb* amorphic mutants. Hatched bars, segments additionally affected when the maternal *hb* complement is lacking¹⁷.

below the limit of detection during stage 14 (data not shown). Thus, the anterior-posterior concentration gradient is probably caused by differential degradation of the maternal *hb* transcripts, as has been proposed for the maternal transcripts of the *caudal* gene^{28,29}.

Zygotic *hb* gene expression was first observed at stage 11. At this stage, transcripts accumulate over the nuclei between 55%–100% egg length (EL) of the embryo (the posterior pole is 0% EL). After an additional nuclear division, transcripts were found in the cytoplasm and in the yolk energids in the area anteriorly adjacent to the domain of *Kr* gene expression. At stage 13, transcripts start to accumulate in a second domain between 0% and 25% EL. In contrast to the earlier anterior domain, the yolk nuclei in this posterior domain remain unlabelled. At stage 14, when cellularization starts, both domains retract from the poles and cover the regions between 10%–25% EL and 55%–90% EL, respectively. Labelling of the posterior domain remains fairly constant from then on. It is then anterior to, and separated by one or two cells from, the *Kr* posterior domain of expression²². In contrast, the anterior domain remains adjacent to the *Kr* domain and undergoes dramatic changes until the beginning of gastrulation (Fig. 3e–h). The overall intensity decreases and it splits up transiently into three subdomains (Fig. 3i and j). At midstage 14, when nuclei begin to elongate significantly, two of these stripes remain as regions of higher

Fig. 5 Sequence of the *hb* region. We have sequenced from the left-hand *Bam*HI site (a genomic *Sau*3A site) and beyond the right-hand *Eco*RI site of Fig. 2. Transcription initiation sites and the intron borders (see below) are indicated by arrows and arrowheads. The presumptive TATA-boxes for the two transcripts, as well as potential polyadenylation signals, are underlined. From Northern blot analysis (see Fig. 2 legend) we infer that the polyadenylation signal at position 7,500 is the one normally used. The underlined amino-acid regions show the conserved protein domains A and B and the boxed regions the two finger domains (compare Fig. 6). Several cDNA fragments were mapped and also partially sequenced. One spans the region between the *Eco*RI site at position 1,246 and position 5,326, excluding the intron. The others lie in the region between 6,267 and the *Eco*RI site at position 7,472. One of them also contains the region between 5,618 to 5,763 artificially ligated in an inverted orientation to position 6,267. The reason for the deletion/inversion is unknown. The sequenced parts of the cDNA clones were identical to the genomic sequence. Full-length cDNA clones were not obtained. The intron borders for the small intron were inferred from the following experiments. Hybridization analysis (Fig. 2) showed that the leader of the 2.9-kb transcript must be located between the *Sal*I site at position 3,940 and the *Xba*I site at position 4,669. *S*₁ mapping with a probe starting at the *Bst*NI site at position 4,735 yields two fragments with a length of 71 and 72 nucleotides (nt). Because the splice acceptor site is 70 nt away, this implies that the nucleotides beyond this should be AG for one transcript and G for the other. The 3.2-kb transcript contains an AG before its splice site, leaving a G for the 2.9-kb transcript. Therefore this donor must contain a GGT motif. Primer extension from an oligonucleotide primer starting at position 4,791 results in two products of equal intensity of 267 nt and 271 nt. Taking the (G)GT motif at position 4,383 as the donor places the transcription initiation sites for the 2.9-kb transcript at two tandem CAGT motifs at positions 4,238 and 4,242, which is close to a consensus TATA-box. Final proof comes from *S*₁-mapping with a probe starting at the *Hae*III site at position 4,372, resulting in two protected fragments of 131 and 135 nt.

Methods. Sequences were determined by dideoxy sequencing⁴⁶ of sonicated fragments which were shotgun cloned in M13 vectors and partly also from overlapping deletions created by DNase I in the pEMBL Vector system⁴⁰. Sequences were determined from both strands throughout the coding region and the 3' end of the intron. Those parts which were sequenced from one strand only (positions 660–830, 2,065–2,510, 2,760–3,310 and 7,472–7,680) were all clearly readable and covered by at least two independent clones. *S*₁ mapping was as in ref. 47.

intensity in the anterior *hb* domain (Fig. 3k). At the beginning of gastrulation, the cephalic furrow³⁰ provides a morphological landmark for those two stripes (Fig. 3l). The posterior stripe extends for 10–12 cells posterior to the cephalic furrow, indicating that *hb* expression has broadened into the thoracic region, now overlapping the *Kr* domain by about six cells (see Fig. 4).

With the beginning of germ band extension³⁰, the concentration of *hb* transcripts in the anterior region falls below the limit of detection, except in the yolk nuclei which remain as intensely labelled as before. With germ-band extension the posterior domain moves dorsally and disappears during the extended germ-band stage, when *hb* transcripts begin to accumulate in the region of the developing nervous system. A comparative analysis with *Kr*, the gap gene that acts posteriorly adjacent to *hb*, is summarized in Fig. 4.

A novel type of finger protein

We have sequenced about 7.7 kb from the *hb* region including the entire transcribed region. The first translation initiation codon (AUG), which is in frame with the main open reading frame, is 3' to the intron and is common to both transcripts (Fig. 5). Consequently, both transcripts encode the same protein with a predicted relative molecular mass of 83,000 (*M*_r). The 3.2-kb transcript contains a single additional AUG codon followed by a short open reading frame in the leader sequence (Fig. 5).

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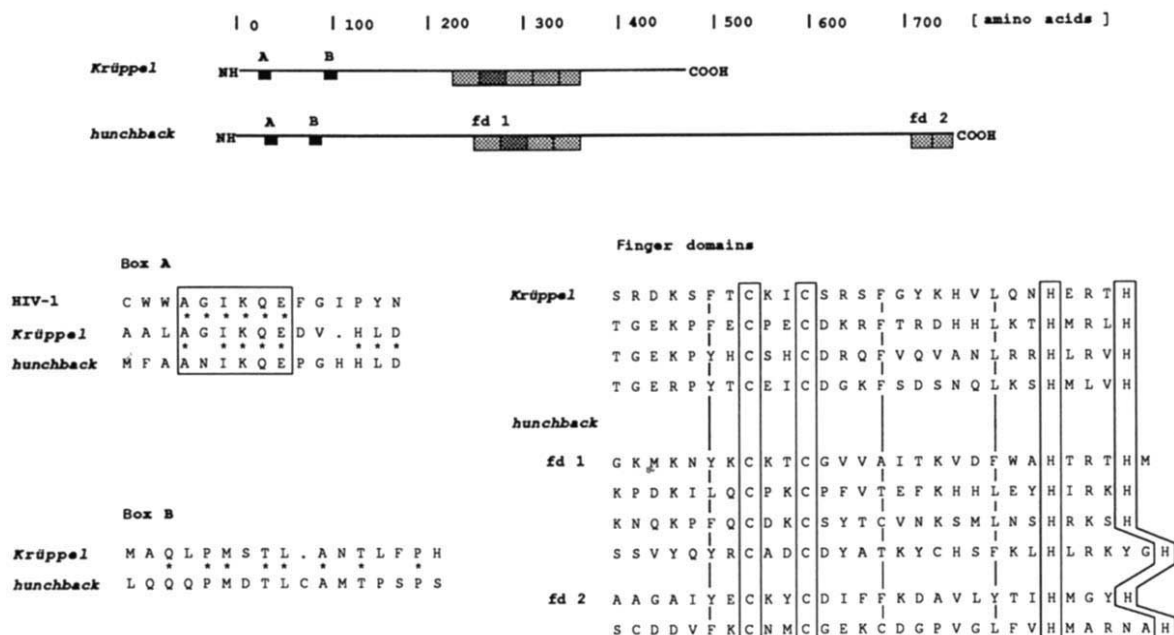


Fig. 6 Structural and sequence similarities between the *Kr* and *hb* proteins. The upper lines show the structural comparison between the two proteins, with the key elements shown in the correct relative positions. The second fingers in both proteins are highlighted, because they are the most similar among the ten fingers. Below this are the direct amino-acid comparisons from the regions indicated above. Box A, the HIV-1 sequence comes from amino-acid positions 857-871 of the endonuclease region of the *Pol* protein⁴⁸, the *Kr* sequence comes from amino-acid positions 16-29 (ref. 23) and the *hb* sequence from positions 22-36. Box B, the sequences are from positions 84-99 in *Kr* and 66-82 in *hb*. For the finger domains, individual fingers of each domain are aligned below each other. For the *Kr* product only the first four complete fingers are shown. The conserved Cys and His residues are boxed, the other conserved positions among finger proteins are indicated by lines.

Analysis of the derived amino-acid sequence predicts DNA binding finger domains in the *hb* protein (Fig. 6). Such domains were first described for the *Xenopus* transcription factor IIIA (TFIIIA)^{31,32} and have since been found in other regulatory proteins including the *Kr* product^{23,33}.

The *hb* fingers contain cysteine, histidine and hydrophobic residues in the appropriate combination and location to fit the finger consensus sequence³⁴. They deviate however from other finger domains in that they contain alanine, cysteine or threonine residues instead of the (normally conserved) phenylalanine residue at the corresponding position in the *Kr* protein (Fig. 6). In contrast to the *Kr* product, the links separating the individual finger domains are variable and only one of them shows similarity to the conserved H-C link motif³⁵ found in the *Kr* protein. In contrast to other finger containing proteins, the *hb* protein shows two distinct finger domains, composed of four and two fingers respectively. The only other example of separated fingers is the *serendipity* gene³⁶ of *Drosophila*. Its product contains a major finger domain composed of several fingers and a single detached finger.

A detailed comparison between the *hb* and the *Kr* protein sequence showed multiple short similarities (search stringency: identity at five out of seven amino acids) which are scattered through the proteins. Two of these, designated box A and box B, are in a similar position relative to the amino terminus in the two proteins (Fig. 6). Strong similarity to the core of box A was also found in the endonuclease region of the *pol* gene of the HIV-1 retrovirus (Fig. 6). The *hb* protein also contains several simple sequence regions which are known to be ubiquitous in eukaryotes^{37,38}. In the *hb* product these are based mainly on a CAN (where N = G, C or A) trinucleotide repeat, frequently found in *Drosophila* coding and noncoding sequences^{37,39}.

Conclusions

The gap genes of *Drosophila* are characterized by deletions of adjacent and partially overlapping segment regions in mutant embryos¹⁰, with *hb* and *Kr* acting in the anterior and the middle

region, respectively. The allelic series of hypomorphic mutants for both genes indicate that the mesothorax is the segment most sensitive to the partial or complete lack of either *hb*⁺ or *Kr*⁺ activity^{17,21}. As shown in Fig. 4, only *Kr* is initially expressed in this segmental anlage²². At the beginning of gastrulation however, *hb* extends into the *Kr* domain and the domains of the two gap genes overlap. Thus, the two gene products subdivide the *hb*-*Kr* phenotypic region into a *hb*, *hb* plus *Kr*, and *Kr* subregions. The *hb*-*Kr* overlap could then serve as a signal for initiation of new gene activities, different from those initiated by each gene alone¹⁶. A requirement for fine tuning of the expression of both genes would explain the sensitivity of the metathoracic segment to the partial lack of either gene.

The maternal *hb* transcript accumulation becomes graded during early cleavage stages, as with the maternal *caudal* transcripts, which form a concentration gradient along the anterior-posterior axis^{28,29} though in the opposite direction to the *hb* gradient. Whereas both maternal and zygotic *caudal* gene products are necessary for embryonal development²⁸, the maternal *hb*⁻ effect is rather limited and can be completely overcome by zygotic *hb*⁺ activity¹⁷. The weak maternal *hb* effect may be related to the autocatalytic activities which are proposed for gap-gene function in Meinhardt's model¹⁶. In this context, the early occurrence and the high initial rate of zygotic *hb* expression could be due to an activating effect of the maternal product, allowing zygotic *hb* expression to be detected earlier than *Kr*, which lacks a maternal product.

Zygotic *hb* expression in the anterior domain is very brief and undergoes several modulations which lead to a transient striped pattern, which could be due to interaction with other segmentation genes. However, preliminary experiments involving hybridization of *hb* DNA to tissue sections of embryos mutant for *fushi tarazu* and *even skipped* (two pair-rule genes) or *engrailed* (a segment-polarity gene) did not result in a changed pattern of *hb* expression. This is consistent with the assumed hierarchical interaction scheme among segmentation genes¹¹⁻¹⁶. It remains therefore an open question as to which genes are

responsible for modulation of *hb* activity.

Several segmentation and homoeotic genes share the homoeo box as a potential DNA binding element (refs 40–42 and references therein). This motif for DNA protein interaction is related to the helix–turn–helix domain of the λ repressor gene^{40,41}. In contrast, the two gap genes analysed so far contain finger domains. This second known motif for DNA binding emerged from the analysis of TFIID, a transcription factor of *Xenopus*^{31,32,43} and ADR1, a transcription factor of yeast³³. The *hb* protein contains two separated finger domains (Fig. 6), making it different from other 'finger proteins' and also from homoeo-box-containing genes, which only ever show one DNA binding domain. The two separate domains in the *hb* protein could be a reflection of its two separate molecular functions, namely in the establishment of the segments and in acting like a suppressor of the activities of genes of the bithorax complex (BX-C). On the other hand, the two separate DNA binding domains could provide a basis for the formation of chromatin loops to bring distant regulatory sites together, as was recently

proposed by Ptashne⁴⁴.

The common finger domains in the two analysed gap genes suggest that their equivalent genetic functions are based on common molecular functions. The overall similarities of the two genes could imply a common evolutionary origin for gap genes, for example by ancestral gene duplications. Alternatively, the observed similarities could be the result of convergent evolution and exon shuffling. The molecular analysis of *kni*, the third member of the gap gene class, will provide further insight into the function of finger domains in gap genes and the relevance of the small boxes of sequence similarity.

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Interaction of antibiotics with functional sites in 16S ribosomal RNA

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Chemical footprinting shows that several classes of antibiotics (streptomycin, tetracycline, spectinomycin, edeine, hygromycin and the neomycins) protect concise sets of highly conserved nucleotides in 16S ribosomal RNA when bound to ribosomes. These findings have strong implications for the mechanism of action of these antibiotics and for the assignment of functions to specific structural features of 16S rRNA.

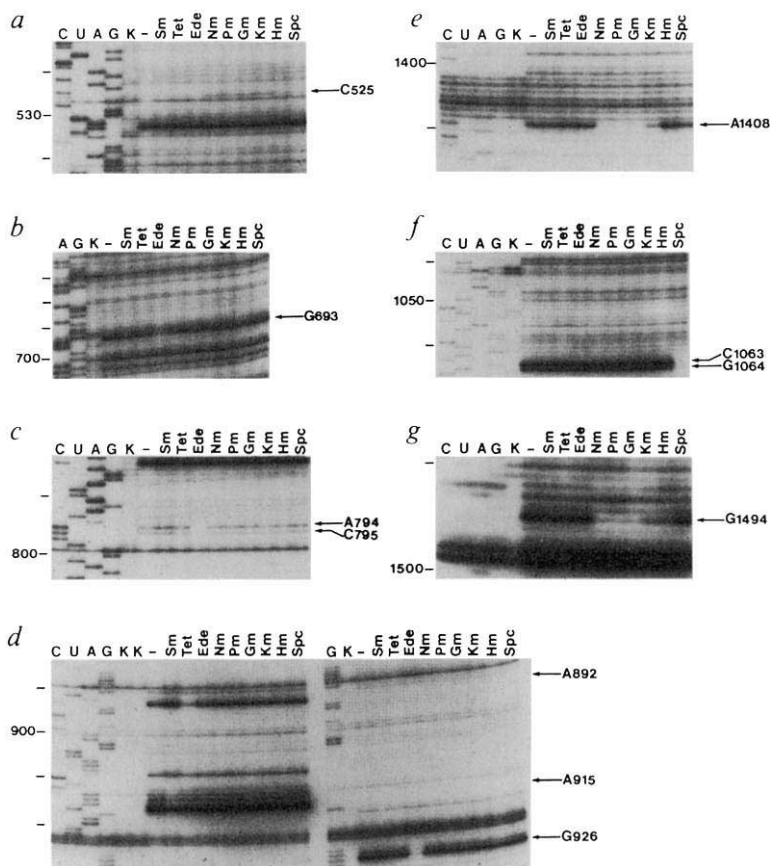
MANY antibiotics exert their effects by interfering with protein biosynthesis, and most of these act directly on the ribosome^{1,2}. Besides their considerable clinical importance, antibiotics of this type have long been recognized as powerful tools in studies aimed at dissecting the mechanism of translation. In spite of a vast literature which correlates the effects of certain antibiotics with perturbation of specific ribosomal events, direct correlation of structure with function has yet to be drawn, because relatively little is known about the actual location of antibiotic binding sites on the ribosome. Until now, the most informative approach has been the isolation of mutants in which antibiotic resistance

is shown to be caused by specific structural alterations in ribosomal proteins or rRNA. Unfortunately, conclusions from such studies are softened by the lack of any convincing demonstration of specific binding of an antibiotic to an isolated ribosomal component.

It is likely that antibiotics exert their effects by binding to functional sites on the ribosome, by analogy with enzyme inhibitors. In keeping with our view that rRNA is the essential determinant of ribosome function, it is reasonable to suspect that many, if not all, antibiotics interact directly with, or otherwise perturb, the structure of RNA. The discovery of numerous

Fig. 1 Autoradiographs showing alteration of reactivity of bases in 16S rRNA toward dimethyl sulphate (DMS) (a, c-g) and kethoxal (b, d) caused by binding of antibiotics to 70S ribosomes. C, U, A and G are dideoxy sequencing lanes and refer to the nucleotide sequence of 16S rRNA³. Sm, streptomycin; Tet, tetracycline; Ede, edeine A1; Nm, neomycin; Pm, paromomycin; Gm, gentamycin; Km, Kanamycin; Hm, hygromycin B; Spc, spectinomycin. K, unmodified RNA; K', unmodified control RNA incubated with streptomycin; -, 70S ribosomes modified in the absence of antibiotic. DMS attacks adenine at N1, cytosine at N3 and guanine at N7; kethoxal reacts with guanine at N1 and N2 (ref. 4). The positions of chemical attack are determined by primer extension using a set of synthetic DNA primers which span the entire 16S rRNA, excluding the extreme 3' end. Reverse transcriptase stops or pauses at modified bases, giving rise to prematurely terminated cDNA chains, whose ends can be located precisely by gel electrophoresis in parallel with dideoxy sequencing lanes generated from unmodified RNA templates. Protections are seen as decreased intensities of the relevant gel bands. In each case, the entire 16S rRNA chain was inspected for perturbation of the modification patterns by each antibiotic. Because of space constraints, data are presented only from those regions of the molecule where significant effects are observed. Arrows, position of major effects.

Methods. Antibiotics were incubated with 100 pmol of ribosomes, prepared as described⁶, in 100 μ l of 80 mM potassium-cacodylate (pH 7.2) 20 mM MgCl₂, 100 mM NH₄Cl, 1 mM DTT, 0.5 mM EDTA for 30 min at 37 °C and then for 10 min on ice. Concentrations were as follows: 5×10^{-6} M Sm, Nm, and Pm; 10^{-4} M Ede, Gm, Km, Hm, and Spc; 2.5×10^{-4} M Tet. Chemical modification was performed by addition of DMS (2 μ l, 1:6 dilution in EtOH) or kethoxal (5 μ l, 37 mg ml⁻¹ in double distilled water) followed by incubation at 37 °C for 10 min. Reactions were stopped, RNA extracted, and resuspended as described⁶. Primer extension and gel electrophoresis were performed as described previously^{5,6}. Aniline-induced strand scission⁴⁶ was performed for samples in f and g.



mutations in rRNA that confer antibiotic resistance is consistent with this view (for review see ref. 3). Recently, we have developed chemical probing methods which allow us to monitor ligand interactions and conformational changes involving individual bases of RNA with relative ease^{4,5}. If an antibiotic binds rRNA by stable interactions with specific nucleotide bases, it should be possible to identify such sites by their diminished reactivities toward our probes. Here, we use this approach to examine several of the most intensively studied antibiotics that bind to the small ribosomal subunit, including streptomycin, tetracycline, spectinomycin, hygromycin, edeine, and several members of the neomycin group (neomycin, paromomycin, kanamycin and gentamycin). In every case, a restricted set of bases in 16S rRNA is shielded from chemical attack in the presence of the bound drug. All the most strongly protected bases are phylogenetically conserved and are located in parts of the 16S rRNA structure that have been implicated previously in ribosomal function. Moreover, we observe striking correlations between the locations of the protected bases and those which are altered in antibiotic-resistant mutants, where such data are available.

Besides providing important clues to the mode of action of these antibiotics, our findings suggest connections between certain structural features of 16S rRNA and ribosome function. In particular, sites protected by antibiotics are closely correlated with those protected by transfer RNA⁶. Nucleotides protected by tRNA (Fig. 4) can be classified operationally according to whether their protection is strictly mRNA-dependent (class I) or mRNA-independent at high Mg²⁺ concentrations (class II), or whether they are also protected by 50S subunits, in the absence of tRNA (class III). Earlier, we predicted that classes I and II are likely to correspond to A-site and P-site binding of tRNA,

respectively⁶. Our present findings support this prediction, and shed light on the nature of class-III sites. The results of our probing experiments are shown in Figs 1 and 2 (only the relevant sections of the gels are presented). In the following, we discuss separately the results for each antibiotic.

Streptomycin

Streptomycin is probably the most extensively studied ribosome-directed antibiotic. Its main effects on translation are to induce misreading of the genetic code and to inhibit translational initiation^{7,8}. Streptomycin-induced miscoding is believed to be the result of interference with a proof-reading step in translation, rather than with the initial step of tRNA selection⁹⁻¹¹. Thus, localization of the site of action of this drug may identify structural features of the ribosome that are involved in proof-reading, one of the fundamental steps in the process of translation. In *Escherichia coli* the three main streptomycin phenotypes—sensitivity, resistance and dependence—were originally shown to be governed by ribosomal protein S12 (refs 12 and 13). Streptomycin-dependence can be suppressed by mutations in protein S4 (*ram* mutants) which, when present in a streptomycin-sensitive background, cause increased misreading (ribosomal ambiguity)¹⁴. More recently, mutants have been obtained in which streptomycin resistance is conferred by a C to U transition at position 912 of 16S rRNA (*E. coli* numbering)^{15,16}. This result points to the possibility that the target site of streptomycin is 16S rRNA.

When streptomycin is bound to 70S ribosomes, we observe strong protection of adenosines 913-915 from attack by dimethylsulphate (DMS) and weak, but significant, protection of U911 and C912 (Fig. 1d, Sm lane). The site of protection of 16S rRNA by streptomycin thus surrounds and includes the

Fig. 2 Chemical probing experiments showing protection of bases in 16S rRNA caused by binding of antibiotics to 30S subunits. Subunits were activated by heating for 30 min at 40 °C in modification buffer. Binding and modification were then performed as in Fig. 1 legend. *a*, DMS (left) and kethoxal reactions (right); *b*, DMS reactions and *c*, kethoxal reactions from the relevant regions of the molecule.

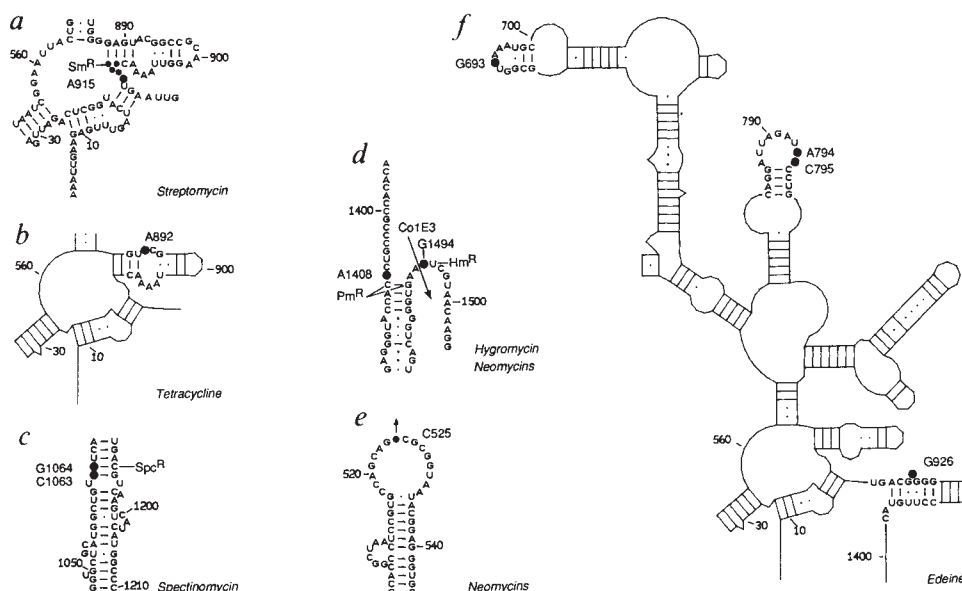
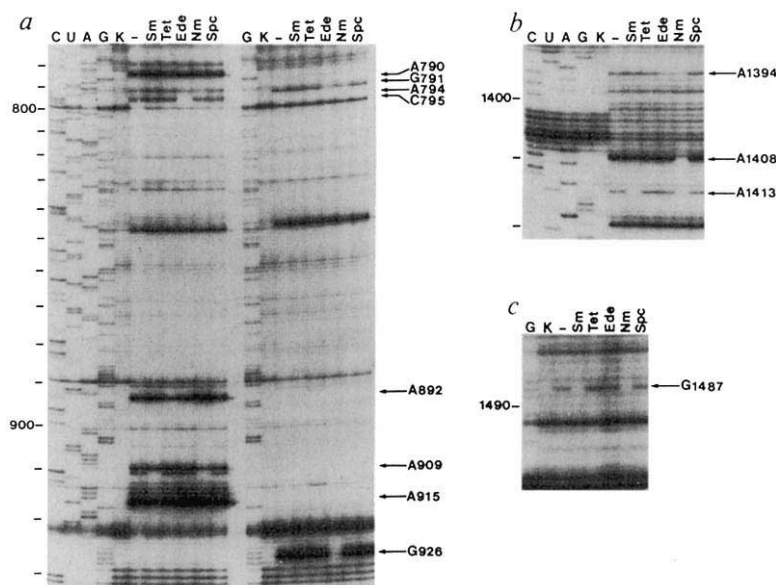


Fig. 3 Regions of *E. coli* 16S rRNA (*a-f*) showing positions where binding of antibiotics affects reactivities of bases (●). Reactivity of C525 is enhanced; all other effects are protections, except for A1,408, which is enhanced by hygromycin but protected by neomycin-class antibiotics (see Table 1). Sites of mutations conferring drug resistance, the site of cleavage by colicin E3, and C1,400, the site of crosslinking of the anticodon of tRNA to 16S rRNA, are indicated (see text for references).

previously identified base (C912) in which mutation confers streptomycin resistance (Fig. 3*a*). Reactivities of the other bases in 16S rRNA are unaffected by the drug.

This agreement between the results obtained from biochemical and genetic approaches implies that the primary site of action of streptomycin includes the 915 region of 16S rRNA. This region is at the confluence of the three major secondary structural domains, and so in a sense lies at the hub of the 16S rRNA structure (Fig. 4). A915 is adjacent to the three base pairs formed by residues 17–19 and 916–918 (Fig. 3*a*), which form the single pseudoknot structure found so far in a ribosomal RNA¹⁷. Flanking the 5' side of the streptomycin-protected sequence is a helix (888–891/909–912) whose existence is in question¹⁷. The low chemical reactivity of bases here⁴ implies involvement in higher order structure of some kind, although this may yet turn out to be tertiary or quaternary. Included in this region is A909, a class-III tRNA protection site (Fig. 4)⁶. Finally, the streptomycin site is midway between the decoding region around position 1,400 (ref. 18) and the binding site for protein S4, which is centred at the junction of five helices around position 500 (ref.

19). Because S4 *ram* mutants have been shown to affect proof-reading^{20,21}, the streptomycin site may be part of a structural link between regions of the ribosome that are involved in codon-anticodon interaction, and proof-reading, respectively⁶. Another possibility is that the streptomycin site and the 1,400 region are actually proximal, as discussed below.

Tetracycline

Inhibition of protein synthesis by tetracycline is generally believed to be the result of interference with binding of aminoacyl-tRNA to the ribosomal A site^{2,22–24}. Ribosomal mutations conferring tetracycline resistance are difficult to isolate, mainly because of the frequent occurrence of mutants whose cells are impermeable to the drug. Somewhat more is known about the location of the A site. Because the anticodon of P-site-bound tRNA can be crosslinked directly to C1,400 (ref. 18), and that of A-site-bound tRNA can be cross-linked to the same position by a 23-Å linker^{25,26}, the anticodon of bound aminoacyl-tRNA must be spatially proximal to the 1,400 region. Consistent with this is the finding⁶ that A1,408, A1,492 and

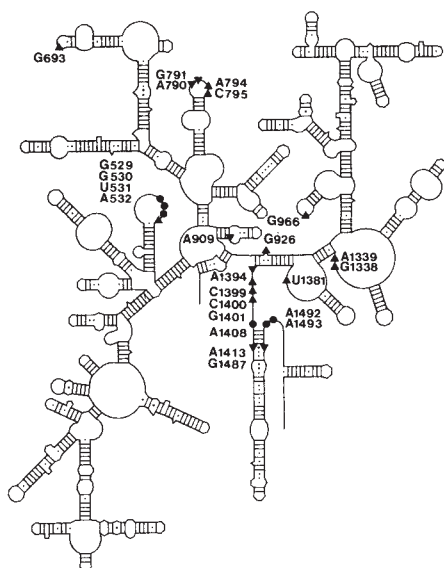


Fig. 4 Diagram of the secondary structure of *E. coli* 16S rRNA, summarizing sites protected by tRNA from chemical probes (ref. 6 and D.M. and H.F.N., unpublished). ●, Class-I (mRNA-dependent) protections; ▲, class-II (mRNA-independent) protections; ▼, class-III (tRNA or 50S subunit) protections.

A1,493 are class-I tRNA protection sites (Fig. 4). Further support comes from the fact that colicin E3 cleavage at A1,493 is blocked by bound aminoacyl-tRNA and by tetracycline²⁷.

In view of this, it is perhaps surprising that tetracycline has no detectable effect on the probing pattern in the 1,400–1,500 region. Instead, we observe strong protection against DMS modification of A892 (Fig. 1d), proximal to the site protected by streptomycin (Fig. 3b). This immediately suggests a structural connection between A-site binding and proof-reading and raises the possibility that the tertiary folding of 16S rRNA might bring the 890 and 1,400 regions into close proximity. Tetracycline also causes significant enhancement of the reactivities of U1,052 and C1,054, at a seemingly remote location in the 3' major domain. The possibility that more than one molecule of tetracycline binds to the 30S subunit cannot yet be excluded², so this last effect may be attributable to binding at a second site.

Spectinomycin

The bacteriostatic action of spectinomycin is thought to be due to inhibition of translocation of peptidyl-tRNA from A site to P site during early rounds of peptide bond formation^{28,29}. Spectinomycin resistance has been shown to be conferred by mutations in ribosomal protein S5 (ref. 30) and, more recently, by a C to U transition at position 1,192 in the 3' major domain of 16S rRNA³¹.

Spectinomycin strongly shields C1,063 and G1,064 from DMS (Fig. 1f). One site of attack of DMS can be assigned to N7 of G1,064, but interpretation of the C1,063 modification is less straightforward. Typically, DMS reacts with an unpaired C at its N3 position, and the resulting ³mC is readily detected as a reverse transcriptase stop. In the case of C1,063, a stop is not observed unless an aniline-induced strand cleavage is first performed (data not shown). We cannot exclude the possibility that spurious cleavage at C1,063 may occur by some combination of the aniline treatment and the presence of the adjacent ⁷mG at position 1,064.

In any case, the site of chemical protection by spectinomycin bears an interesting relationship to the genetic result. Although position 1,064 is some distance from the base conferring resistance at position 1,192, these same two nucleotides are base-paired with one another in the secondary structure of 16S rRNA (Fig. 3c). Because a C to U mutation at 1,192 would transform a G-C pair into a G-U wobble pair, it is reasonable to suspect that the resulting distortion of local geometry could disrupt specific contacts between the drug and the rRNA. How this region of 16S rRNA might be involved in the translocation process remains a mystery. Finally, we note that spectinomycin

also causes detectable enhancement of DMS modification of G973 at its N7 position (data not shown).

Hygromycin

Aminoglycoside antibiotics are believed to exert a dual effect on protein synthesis. Like streptomycin, they induce misreading, although in a fashion that is distinguishable from streptomycin-induced misreading³². At the same time, they inhibit translocation, apparently by sequestering peptidyl-tRNA in the A site^{33,34}. Among the aminoglycosides, hygromycin is especially useful for studies on structure and function, because it has a monophasic effect on protein synthesis³⁵. Accordingly, its action is probably due to binding of a single drug molecule. In support of this, one-step mutations to hygromycin resistance have been found in *Tetrahymena*³⁶, caused by a U to C transition at a position in 18S rRNA corresponding to U1,495 in the *E. coli* 16S rRNA numbering scheme (Fig. 3d).

Hygromycin protects N7 of G1,494 from attack by DMS (Fig. 1g) and causes significant enhancement of modification of A1,408 at N1 (Fig. 1e). Here again, the site of protection by an antibiotic is intimately juxtaposed with the site of mutation conferring resistance (Fig. 3d). In this case, both are proximal to the decoding region, suggesting a structural basis for hygromycin-induced misreading. Because A1,408, A1,492 and A1,493 are class-I tRNA protection sites⁶ (Fig. 4), the dual effects of hygromycin on protein synthesis could be explained as a distortion of the conformation of the ribosomal A site in the decoding region. This is reinforced by results obtained with antibiotics of the neomycin class in the following section.

Other aminoglycosides

The structurally related aminoglycosides neomycin, paromomycin, gentamycin and kanamycin also inhibit translocation and elicit miscoding^{33,34,37}. Paromomycin resistance has been shown to result from mutations that give single base changes in 15S rRNA from yeast mitochondria³⁸, or 18S rRNA from *Tetrahymena*³⁶. The former has an alteration at position 1,409, and the latter at position 1,491 (numbered according to *E. coli* 16S rRNA); in either case, the result is to disrupt the position 1,409/position 1,491 base pair (Fig. 3d). The resulting mispair is directly adjacent to the same three class-I tRNA protection sites (Fig. 4).

All four of these aminoglycoside antibiotics have a qualitatively similar effect on the chemical reactivity pattern. There is very strong protection of A1,408 at N1 (Fig. 1e) and G1,494 at N7 (Fig. 2g); protection of both sites is less complete with kanamycin than for the other three drugs. These effects resemble

Table 1 Protection of specific bases in *E. coli* 16S rRNA by antibiotics

Antibiotic	Binding to 70S ribosome		Binding to 30S subunits	
	Strong	Weak	Strong	Weak
Streptomycin	A913, A914, A915	U911, C912	A913, A914, A915	U911, C912, A909, A1,413, G1,487, G1,494
Tetracycline	A892	U1,052†, C1,054†	A892	U1,052†, C1,054†
Spectinomycin	C1,063, G1,064	G973†	C1,063, G1,064	G973†
Hygromycin	G1,494	A1,408†	—	G1,494, A1,408†
Neomycin	A1,408, G1,494	C525†	A1,408, G1,494	C525†
Paromomycin				A790, G791, A909,
Gentamycin				A1,394, A1,413, G1,487
Kanamycin				A790, G791, A1,394
Edeine	G693, G926, A794, C795	—	G693, G926, A794, C795	

Data from chemical footprinting experiments with 70S ribosomes and 30S subunits for the different antibiotics, according to whether they show strong or weak protection from visual inspection of the autoradiograms is summarized. The chemical reaction specificities of U911, U1,052 and C1,063 are unusual and not presently understood. Neomycin also shows weak enhancement of A65 and weak protection at N7 of G833, G836, G849 and G851 in 30S subunits. Gentamycin and kanamycin also give weak protection at N7 of G832, G833, G836 and G849 in both 30S subunits and 70S ribosomes. In addition, kanamycin weakly protects G851 and C862, and weakly enhances G973. † indicates enhancement.

those observed with hygromycin, except that whereas A1,408 is enhanced by hygromycin, it is strongly protected by antibiotics of the neomycin class. Furthermore, the four neomycin-related drugs induce significantly enhanced reactivity at C525 (Fig. 1a). Various additional weak effects are observed with the neomycin group, although these tend to be idiosyncratic. Kanamycin causes enhancement at the N7 position of G973, whereas both gentamycin and kanamycin give weak protection of several bases in the 850 region (data not shown; see Table 1). Multiple ribosome binding sites have been reported for this class of antibiotics². At the low concentrations of drug used in our experiments, we expect to approach full occupancy only at the strongest binding site; because the inhibitory effects on protein synthesis are also observed at similar low antibiotic concentrations, we infer that the strongly protected sites are relevant to the physiological action of the drug, and that some of the weaker effects may be due to secondary binding sites. The weak protections in the 850 region are likely to be of this type, in keeping with the phylogenetic variability of this part of 16S rRNA. Paromomycin, which has been shown to have a single ribosome binding site³⁹, shows no effects in the 850 region.

Antibiotics of the neomycin class reiterate the pattern of occurrence of a strongly protected site, in this case A1,408, directly adjacent to the site of mutation conferring drug resistance (the 1,409/1,491 base pair; Fig. 3d). As for hygromycin, a plausible model would be that the neomycins interfere with the decoding region of the A site. It is clear, however, that the structural effects of these two classes of antibiotics differ in

detail. The enhancement of C525 recalls the protections observed in the 530 loop induced by mRNA-dependent binding of tRNA or its anticodon stem-loop⁶. Structural considerations make it seem unlikely that protection of the 530 loop is caused by direct contact; accordingly, we have considered the possibility that it is the result of an induced conformational change, transmitted in some way from the decoding region to the 530 loop. By analogy, the observed enhancement of C525 could also be explained by an allosteric change induced by binding of neomycin-class aminoglycosides to the decoding region of the A site.

Edeine

Under the conditions used in our experiments, the oligopeptide antibiotic edeine is believed to block P-site binding of tRNA⁴⁰. Thus, it complements the A-site-specific drugs described above and has been used in earlier studies to distinguish between A-site and P-site binding⁴¹. It causes strong protection of both G693 and G926 from kethoxal (Fig. 1b and d) and of A794 and C795 from DMS (Fig. 1c). All four of these bases are class-II tRNA protection sites (Fig. 4). This, along with the previously established functional specificity of edeine, confirms our earlier prediction⁶ that class-II sites are protected by P-site-bound tRNA. Finally, if these effects are due to direct contact with a single molecule of edeine, positions 693, 794, 795 and 926 must be in close proximity to one another in the ribosome. Crosslinking of positions 693–696 to position 794 by *bis*-2-chloroethylamine⁴² confirms the proximity of positions 693, 794 and 795.

Effects of antibiotics on 30S subunits

When similar experiments are carried out on isolated 30S subunits, the same effects are observed as for 70S ribosomes, but some of the drugs show additional 30S-specific protections (Table 1). In almost every case, these additional protections occur at nucleotides that are unreactive in 70S ribosomes. Furthermore, a characteristic subset of nucleotides (A790, G791, A909, A1,394, A1,413 and G1,487) tend to be represented in the 30S-specific protections for several antibiotics. Interestingly, this subset corresponds precisely to the class-III tRNA protection sites (Fig. 4). The neomycin class protects all six of these sites, whereas streptomycin protects half of the subset (909, 1,413 and 1,487) and edeine protects the other half (790, 791 and 1,394; Fig. 2; Table 2).

It is intriguing that three structurally and functionally diverse groups of antibiotics (streptomycin, the neomycins and edeine) all protect members of this same subset of nucleotides, although at least two of these three classes of drug (streptomycin and neomycin) fail to compete with each other for ribosome bind-

Table 2 Protection of class III tRNA protection sites by antibiotics

Position	Protecting ligand		
	50S subunits or tRNA	Neomycin	Streptomycin
A 790	+	+	—
A 791	+	+	—
A 909	+	+	+
A 1,394	+	+	—
A 1,413	+	+	+
G 1,487	+	+	+

Protection by 50S subunits or tRNA is from ref. 6 and D.M. and H.F.N. (unpublished results). Symbols: +, protected; —, not protected by the corresponding ligand, when bound to 30S ribosomal subunits. Class-III sites are defined in the text.

ing³⁹. Taken together with the fact that these class-III sites are also protected by tRNA or 50S subunits, which are also known not to compete with each other in binding to 30S subunits, it seems unlikely that protection of class-III sites by any of these ligands occurs by a simple, direct mechanism. Two alternative, unifying models would be either that tRNA, 50S subunits or antibiotics can induce the same conformational change in 30S particles (although the antibiotic results imply that at least two separate structural changes are involved), or that a solvent-inaccessible pocket is formed between the 30S and 50S subunits, which can also be blocked by tRNA or drugs. If the former explanation is correct, it immediately suggests how certain antibiotics could perturb the accuracy of translation. The nature of class-III sites clearly warrants further study.

Conclusions

Comparative analysis has revealed the existence of regions of universally conserved nucleotide sequence in ribosomal RNA in a framework of highly conserved secondary structural elements^{17,43}. These sequences must therefore be among the most ancient that exist on our planet. Such extreme conservation implies that these sequences are somehow crucial for the translation process; most probably, they help to form RNA 'active sites'. The results of biochemical and genetic experiments tend to support this notion, and have begun to suggest the functions of these sites³. The protection of individual nucleotides by specific antibiotics, whose effects on protein synthesis have been well studied, provides new information of this kind. Except for the intriguing case of the class-III sites, we make the simple working assumption that strongly protected nucleotides represent sites of contact between 16S rRNA and the antibiotic. This assumption is borne out by a recurrent theme—the close proximity of these sites to nucleotides whose mutation gives rise to antibiotic resistance, or to nucleotides that have previously been implicated in specific ribosomal functions. We note a general correlation between the phylogenetic range of drug sensitivity² and the phylogenetic conservation of protected nucleotides⁴³. Thus, universal ribosome inhibitors (such as tetracycline, hygromycin and edeine) protect universally conserved bases, whereas group-specific antibiotics (such as streptomycin and spectinomycin) tend to protect nucleotides that are more phylogenetically variable (C912, G1,064).

In the cases of streptomycin^{15,16}, spectinomycin³¹, paromomycin³⁶ and hygromycin³⁶, mutations in small subunit rRNA genes

have been identified that confer resistance to these drugs. In every case, binding the corresponding antibiotic to 70S ribosomes causes strong protection of nucleotides immediately adjacent to the resistance site. In studies on binding of tRNA to ribosomes we identified G693, A794, C795 and G926 among those nucleotides protected from chemical probes by tRNA in a mRNA-independent fashion at high concentrations of Mg²⁺ (class-II sites). Accordingly, we suggested that protection of these sites is due to P-site binding of tRNA. Here, we show that these same positions are protected by edeine, a P-site inhibitor. Similarly, other results presented here support our previous assignment of class-I sites to protection by A-site-bound tRNA.

Colicin E3 inhibits protein synthesis by cleaving a single phosphodiester linkage between A1,493 and G1,494 of 16S rRNA, in 70S ribosomes^{44,45}. The action of colicin E3 is prevented by bound aminoacyl-tRNA and by streptomycin, tetracycline or gentamycin²⁷. It is not difficult to imagine how aminoacyl-tRNA or gentamycin might accomplish this, because both of these ligands protect nucleotides in this region (tRNA, presumably A-site bound, protects positions 1,492 and 1,493; gentamycin, position 1,494). However, chemical protection by streptomycin and tetracycline is localized to positions 892 and 915, respectively. If their inhibitory effect on colicin E3 is a result of direct interference with the interaction of the colicin with its cleavage site, it would require the 900 and 1,490 regions of 16S rRNA to be proximal in the ribosome, and would help to explain the physiological effects of these antibiotics. Weak protection of G 1,494 at its N7 position by streptomycin (Table 1) hints at this possibility.

Apart from the Shine-Dalgarno mechanism for mRNA selection, we currently have no clearly defined model for how rRNA participates in translation. This may owe as much to limitations in our knowledge of the catalytic and other physicochemical capabilities of globular RNAs, as to lack of specific data pertinent to rRNA. The results presented here would argue that functional sites in 16S rRNA tend to be made up of a 'three-dimensional mosaic' of individual nucleotides distributed among several conserved regions of the RNA structure. Most probably, a better understanding of the higher-order folding of these features will be required before we have a clear description of the workings of rRNA.

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Physical process which shapes Cassiopeia A

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The Cassiopeia A nebula provides a special opportunity for improving our understanding of how massive stars explode since it is the remnant of the most recent instance (AD 1680) of such a supernova event (of which we are aware) within our own Galaxy. Its relative proximity (a distance of 2.9 kpc) makes it possible to study the source's structure and time evolution in sufficient detail and over a sufficiently broad spectral range (from X-rays to radio waves) that the important physical processes are becoming increasingly well-defined. Here we report how we have exploited the rapid time evolution of the source through a series of three high-resolution radio images spanning four years. These images have allowed us to recognize the previously unsuspected process which is in fact dominant in shaping the object. Tenuous clumps of intermediate velocity stellar ejecta are now continuously puncturing, from within, the decelerated shell swept up by the diffuse ejecta which once had the highest velocity.

Although the Cas A event was not recognized during its early history, its discovery¹ in 1948 as the most prominent radio source in the sky initiated a period of intensive investigation. Its bright radio emission is now known to originate in a complex shell where supernova ejecta collide with the swept-up interstellar medium. Studies of extragalactic Type II supernovae show that a large amount of material is ejected ($\sim 5 M_{\odot}$) with a wide range of velocities up to a maximum of $\sim 10,000 \text{ km s}^{-1}$, but most of the energy is probably deposited in the small mass fraction with highest velocity. The most energetic component will generate a blast wave in the interstellar medium which then decelerates as it sweeps up more material, allowing the more slowly moving, massive component to overtake it. This leads to the formation of a turbulent shell and the propagation of the reverse shock back towards the site of the supernova. The general picture outlined above is supported by observations of the nebula made throughout the electromagnetic spectrum.

A self-consistent analysis of previous observations has recently been published (in ref. 2), of which the most salient points are reiterated here. The geometry of the expanding shell is well-determined by observations of the fast-moving optical knots (dense clumps of stellar ejecta which are shock-heated and recombine as they penetrate the shell). The radial velocities of this population indicate the first-order physical model shown in Fig. 2a; a smaller hemispherical front face is attached to a larger hemispherical rear face by a flaring region which is more

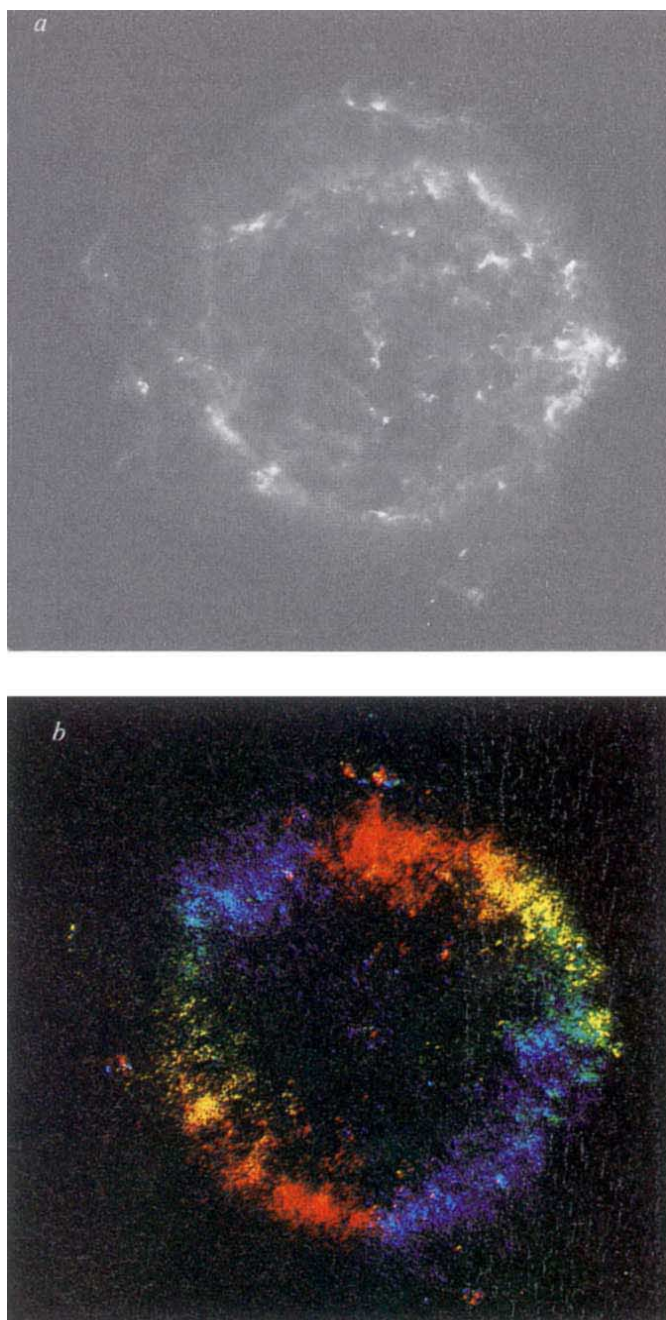


Fig. 1 *a*, Radio image of Cas A at epoch 1983.8. The angular resolution is 0.4 arc s, while the bright ring of emission has ~ 240 arc s diameter. Careful examination of this image reveals the population of turbulent clumps, enclosed within the paraboloid extensions sketched in Fig. 2*b*. *b*, The intensity of linearly polarized radio emission from Cas A (at epoch 1983.8 with 1.3 arc s resolution) is colour-coded in this image by the projected magnetic field orientation. The predominantly radial field orientation is shown by the systematic change in colour from blue to green, yellow, red and back to blue on the opposite side. Local departures from this pattern indicate regions of tangential and turbulent field orientation. Every local departure seen corresponds to the apex of one of the extensions sketched in Fig. 2*b*.

Table 1 Summary of VLA observations

Date	VLA configuration ⁷	Observing frequencies (MHz)	Duration (h)
1981.1	A	1441	11.5
1983.0	C-D	1465	6.0
1981.6	B	4862	9.5
1983.0	D	4885	8.5
1983.8	A	4410, 4640, 4970, 5085	9.5
1984.0	B	4640, 4970	9.5
1985.2	A	1381, 1456, 1536, 1626	9.0
1985.3	B	1381, 1626	9.0
1985.2	A	4410, 4640, 4970, 5085	9.0
1985.3	B	4640, 4970	9.0

or less in the plane perpendicular to our line of sight. The analysis of shock-heated dust emission observed with the Infrared Astronomical Satellite, together with the overall source expansion observed in previous radio imaging has allowed a good estimate to be made of the physical conditions in and around the source. Only a relatively small mass ($0.3 M_{\odot}$) of

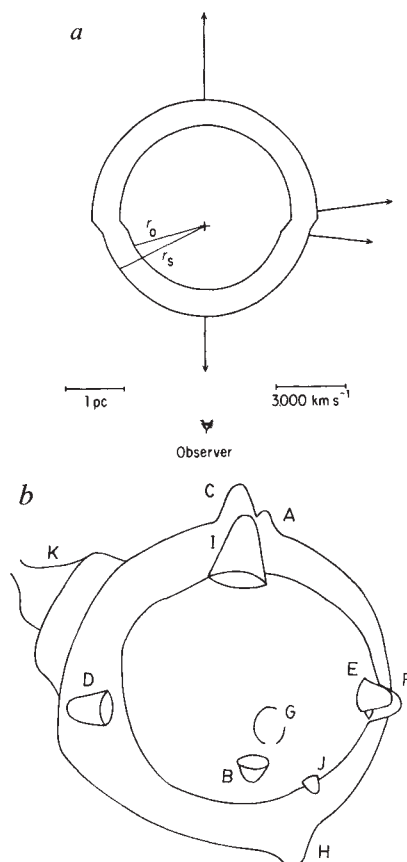


Fig. 2 *a*, First-order geometrical model of Cas A viewed from above the galactic plane, as derived from optical and radio dynamical data (see ref. 2). The vectors indicate the current velocity of the outer shock, while the surfaces at r_0 and r_s represent the locations of the reverse and outer shocks respectively. *b*, Sketch of major shell and bow-shock features identified in the current program of radio imaging. The limb-brightened reverse shock interface (r_0 in Fig. 2*a*) of the smaller front hemisphere is seen in projection against the rear hemisphere. Paraboloid extensions labelled A-K are visible as they propagate in the zone of enhanced particle and energy density between this surface and the outer shock (at r_s).

diffuse ejecta has now swept up $1.3 M_\odot$ of diffuse gas in the source's environment. Pre-shock densities which are now being encountered range from $n_H = 4$ to 0.4 cm^{-3} , while the initial shock velocity of $\sim 11,000 \text{ km s}^{-1}$ has slowed to the range $v_s = 2,300$ to $3,900 \text{ km s}^{-1}$ for the front and rear shell faces respectively. This relatively low density environment is likely to correspond to the interior of the cavity within the parent molecular cloud blown by the energetic stellar wind of the supernova progenitor. Once the dense cavity walls are encountered (within a few thousand years) a rather different phase in the source's evolution will begin; marked by shock-accelerated neutral and molecular gas and the optical forbidden line filaments typical of such supernova remnants³.

Previous radio observations of Cas A (epochs 1969.9⁴ with 6 arc s resolution, 1974.9⁵ and 1978.9⁶ with 2 arc s resolution) have revealed a wealth of complex structure, proper motions and changes in brightness of compact features. However, discerning changes in shape and brightness from changes in position for the many unresolved features is still limited by the available spatial resolution. Moreover, the significant variations in brightness over the five year intervals separating these previous observations has made it difficult to identify and track many of the more diffuse features. The potential for a better understanding of this class sources, based on more adequate spatial and temporal resolution of the evolving radio brightness

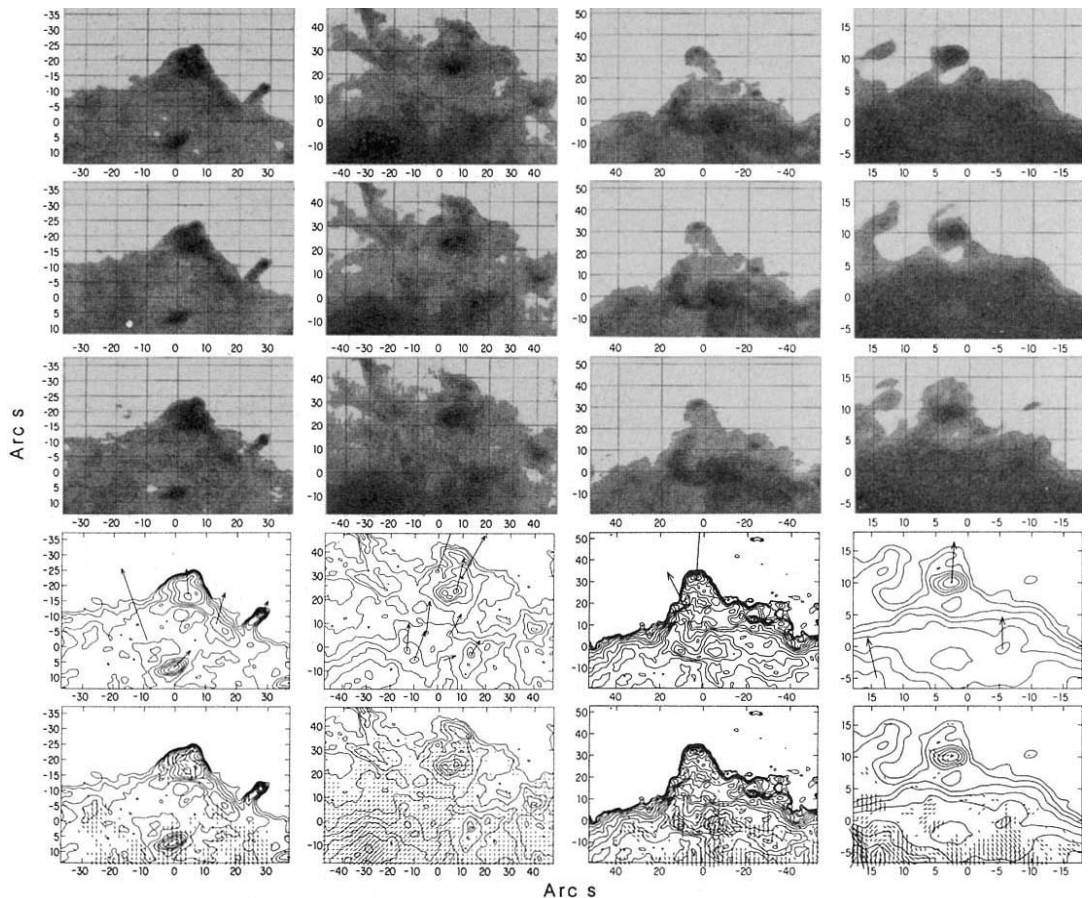
Table 2 Bow-shock parameters

Feature	Minimum μ_{obs}	Ellipticity	Estimated μ_0	Mach number
A	28°	~ 0	28°	2.1
B	22.5°	0.68	17°	3.4
C	26°	~ 0	26°	2.3
D	11.5°	0.43	10.5°	5.5
E	20°	0.38	18.5°	3.1
F	20°	0.25	19°	3.1
G	—	~ 1	—	—
H	31.5°	~ 0	31.5°	1.9
I	20.5°	0.29	19.5°	3.0
J	13°	0.29	12.5°	4.6
K	21.5°	~ 0	21.5°	2.7

distribution of Cas A, has motivated our current programme of observations with the Very Large Array (VLA)⁷. High-quality, multi-configuration data with 1.3 arc s resolution were obtained in 1981.1, 1981.6 and 1985.2, and with 0.4 arc s resolution in 1983.8 and 1985.2. Aperture coverage was improved in the case of the higher-resolution configurations by combining observations made over a range of frequencies, spanning about 10% of the nominal frequency. Details of these observations are summarized in Table 1. Deconvolution of the high-resolution epoch 1983.8 data was carried out on a $4,096 \times 4,096$ pixel grid via a maximum entropy method algorithm⁸. This processing was accomplished with 20 minutes computer processing time on the Cray X-MP at Digital Productions in Los Angeles through the National Science Foundation supercomputer access program. The result represents the largest and most detailed radio image yet obtained.

While reduction and analysis of this database is only partially complete, a clearer indication of the important physical processes has already begun to emerge. A careful examination of the epoch 1983.8 image in Fig. 1*a* reveals that virtually all of the emission is due to two distinct, yet related, types of structure in various stages of development. The basic form is of a pyramidal structure—each of which has a turbulent region at its apex and a trailing paraboloid sheath. The clearest examples of such structures have been sketched and labelled in Fig. 2*b* to ease their identification in Fig. 1*a*. A three-frame time sequence as well as contour maps with proper motion vectors and magnetic field orientation superposed is shown in Fig. 3 for some of the features labelled in Fig. 2*b*. The morphology of these structures is already suggestive of high-velocity clumps that generate a bow shock as they pass through the material of a more slowly-expanding shell, but at least three other lines of evidence give strong support to such a model. The proper motions discernible in this time sequence and indicated in the bottom panel of Fig. 3 show that, while each apex has a primarily radial motion, the sheath walls have large tangential components of motion directed away from the apex. Furthermore, the radial magnetic field alignment which has been recognized in earlier studies⁹ is prominent within the sheath walls (as might be expected under the conditions of stretching suggested by the morphology) whereas the apices show a more tangential and in some cases vortical field. This is strikingly illustrated in Fig. 1*b* where the intensity of linearly polarized intensity from the entire source has been colour-coded with the projected magnetic field orientation. The overall radial field alignment is seen as the regular variation of colour around the source periphery from blue to green, yellow, red and back to blue. There are relatively few instances of local tangential fields as shown by local departures from the regular pattern. Each of those observed corresponds to the apex of one such feature. Finally, the apices which show the least excursion from the inner shell surface are consistently observed to be brightening in radio emission, while the more extended ones are associated with brightening sheaths, although they themselves are fading.

Fig. 3 Evolution and magnetic field alignment within some of the features labelled in Fig. 2b. Each column contains from top to bottom: three grey-scale images at epochs 1985.2, 1983.8, and 1981.1 with 1.3 arc s resolution (the epoch 1981.1 image has a significantly higher noise level than the other two), a contour map of the 1983.8 image (contours are logarithmic with an increment factor of 1.5) with proper motion vectors from ref. 6 superposed and the same contour map with magnetic field vectors (with length proportional to the degree of linear polarization and assuming a constant Faraday rotation across the source of -26 deg^9) superposed. The panels from left to right correspond to features H, D, C and A rotated with respect to the expansion centre of the optical knots so that radially out is to the top.



It would seem that the clumps generate radio emission only when they interact with the high-energy-density plasma of the shell and fade completely once they emerge into the more tenuous and benign environment beyond the outer shock. They leave behind them only an expanding 'inverse crater' as evidence of their passage. These events give rise to both the radial magnetic field structure and the significant non-radial though locally systematic motions, which leave the dark voids on the face of the shell.

Another associated phenomenon appears to be fragmentation of the apex-producing clumps upon impact with the inner shell surface. The most dramatic example of this is also seen in Fig. 3 (feature H). The bright compact blob at the end of the trail (apparently helical, as seen in Fig. 1a) seems to be the result of an encounter with the slow-moving optical knot designated R36¹⁰. This feature began brightening rapidly between 1969 and 1974, levelled off through 1981 and is now rapidly fading. The fragment which has given rise to this feature presumably originated in the more extended clump of ejecta responsible for the associated sheath and had a purely radial velocity, $v_r \approx 5,800 \text{ km s}^{-1}$, typical of the optical fast-moving knots in this region. After fragmentation, its velocity seems to be directed $\sim 45^\circ$ away from radial, implying a space velocity $\sim 4,100 \text{ km s}^{-1}$, neglecting additional deceleration within the shell. That could have been the end of the story of this fragment had it not encountered the slow-moving optical knot, a dense circumstellar/interstellar clump recently overrun by the outer shock, where it was severely decelerated to its current velocity of $\sim 1,000 \text{ km s}^{-1}$, leading to the short burst of brightening which we have witnessed. Although this type of dramatic fragment/slow-moving knot encounter is likely to be rather rare, there appear to be a number of other instances (for example, feature G) of the initial splattering of larger clumps.

While the preceding discussion has been chiefly concerned with a qualitative account of the process responsible for the

cellular shell of radio emission, there is more quantitative support for this model. The bow-shock opening half-angles, μ for the features in Fig. 1b are listed in Table 1 together with the corresponding Mach number $M = 1/(\sin \mu)$ corrected for geometrical foreshortening, wherever possible, assuming intrinsically circular gaps in the shell. The features for which an opening angle could reasonably be defined have Mach numbers in the range 1.9 to 5.5. These values are roughly consistent with the range of Mach numbers $M = 1.9$ to 2.5 expected from $M = \Delta v(1/c) = (v_0 - 3v_s/4)(2/v_s)$ (for adiabatic index $\gamma = 4/3$) given the range of shock velocities $v_s = 3,850\text{--}2,330 \text{ km s}^{-1}$ and initial velocities $v_0 = 6,500\text{--}4,700 \text{ km s}^{-1}$ appropriate for the large to small radius portions of the Cassiopeia shell. This expected trend in M is seen by contrasting the features A, C, H and K (with $M = 1.9$ to 2.7) apparently originating in the large radius rear portion of the shell with features B, D, E, F, I, and J (with $M = 3.0$ to 5.5) originating in the smaller radius front face. In particular, feature I appears to be interacting with a region of significantly higher than average density.

The observed variation in magnetic field alignment and strength are also in agreement with recent numerical simulations¹¹ of bow shocks in the same Mach number range. The strength and direction of the poloidal (in this case roughly radial) and the relative strength of toroidal (seen in projection as tangential) field components for an $M = 3$, $\gamma = 4/3$ bow shock are shown in Fig. 4 (from ref. 11). The contours indicate the relative contribution of the toroidal field, while the vectors indicate the strength and direction of the poloidal field. High radial field strengths are seen in alignment with the bow-shock walls, while a compact concentration of tangential field is predicted within a toroid of diameter similar to that of the obstruction responsible for the bow-shock. This latter effect allows for the estimation of the mass and density of the bow-shock producing clumps of ejecta. The region of tangential magnetic field within each of features B, C, D, E, F, H and J has a diameter of roughly

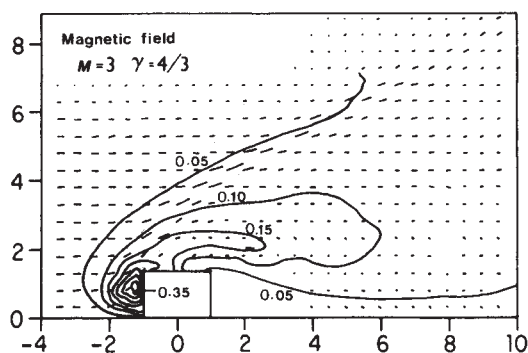


Fig. 4 Post-shock magnetic field configuration for a simulated Mach 3, $\gamma = 4/3$ bow shock induced by a cylindrical obstruction in a supersonic flow (taken from ref. 11). Vectors indicate the strength and direction of the poloidal field and contours represent the ratio of toroidal to poloidal field strength. (See ref. 11 for a complete discussion of the numerical simulation.)

10 arc s corresponding to 0.14 pc (at the 2.9 kpc distance of the source)—the region within feature A being about half this size. Since the shell hydrogen column densities, $N_H = r_c n_c$ lie in the range $0.3\text{--}2 \times 10^{19} \text{ cm}^{-2}$, the shell mass intercepted by the circular area with the above diameter is in the range 0.4 to $2.10^{-3} M_\odot$ for the rear and front shell surfaces respectively. The observed velocity increase of the local shell mass by $\sim 50\%$ implies a clump mass of $\sim 10^{-3} M_\odot$ via momentum conservation. The corresponding density of a spherical clump is $\sim 30 \text{ cm}^{-3}$.

The sensitivity and resolution of the current study have allowed recognition and at least crude quantification of the physical process which seems to be dominant in shaping the radio structure of Cas A. Since the source has evolved to the point where the diffuse shell (that was powered by the initial blast wave) has been significantly decelerated, the more slowly expanding ejecta (presumably from deeper within the star) has begun to break through the shell from within. Fragments of stellar material have apparently condensed after the supernova event. The densest condensations are observed optically as fast-moving knots during their recombination after shell passage. The high density contrast and small linear dimensions of these clumps ensure that they will have little dynamical impact on the shell which shocked them. Larger, more diffuse condensations are much more effectively coupled to the shell during penetration—and it is these which are so dramatically evident in the evolving radio structure. These clumps drive well-defined bow shocks into the shell material, presumably giving rise to local particle acceleration and leave expanding rings of compressed particles and magnetic field in their wake. This process is unambiguously seen in the cases where penetration is not yet complete, while most of the remaining radio structure can be classified as post-penetration rings and voids. The continual recompression by moderate velocity shocks within the shell should lead to relatively low temperature X-ray emission which is roughly correlated with radio brightness, while the dense ridges between voids should act as more effective screens for the population of optical fast-moving knots and could give rise to apparent filaments in the knot distribution. The X-ray and optical data are consistent with these expectations.

As noted at the outset of this discussion, reduction and analysis of the existing database is only partially complete. Careful analysis and continued monitoring of the evolving structures combined with more pertinent magneto-hydrodynamic simulations of bow-shock development within a shell geometry should allow a refined estimate of the physical parameters. The proximity and rapid evolution of these features may also provide one of the best opportunities to constrain theories of *in situ* particle acceleration. If favourable circumstances allow observations of

other young Type II supernova remnants these would further constrain the distribution of ejecta mass with velocity at the time of the SN event, which in turn would provide a valuable constraint on models of supernovae. Clearly, much theoretical and observational work still remains to be done to remedy the many gaps in our understanding of the supernova phenomenon.

We thank Martin Brown and Richard Tuffs for their assistance in observing, calibrating, organizing and imaging the data as well as Tim Cornwell and Bob Duquet for their help in deconvolving the epoch 1983.8 data. We thank Philip Angerhofer for initiating this project and for his continued interest in it. His premature death has taken from us a most valued colleague.

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Possible models for the high-energy transient GB790107

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The discovery of rapid repetitive soft γ -ray burst events on time-scales as short as seconds from GB790107 by Laros and co-workers and Atteia and co-workers^{1–3} may have important implications for our understanding of the γ -ray burst mechanism. Although there is a general consensus that the high-energy transient phenomenon in the X-ray range known as Type I X-ray bursts can be successfully explained in terms of a thermonuclear flash in the accreted surface layers of a neutron star^{4,5}, the theory for γ -ray bursts is still controversial. In fact, it is possible (perhaps even likely) that there is more than one mechanism by which the γ -ray burst phenomenon can be understood. The properties of GB790107 place severe constraints on the viability of models proposed for the typical γ -ray burst events as applied to this soft γ -ray repeater. Here we review the various models proposed for γ -ray bursts and show that a model involving a comet cloud around a neutron star is consistent with the observational data.

The observed characteristics of the bursts from GB790107 can be briefly summarized as follows^{1–3,6}. They are distinguished from typical γ -ray burst events by their rapid recurrence. Over 100 events have been detected since 1979 and on one occasion (16 November 1983) 16 events were detected with 10 events occurring within one hour. The burst profiles can be characterized as spikes of width less than ~ 0.5 s with a typical rise and decay time ranging from 0.01–0.1 s. The burst spectra are much softer than typical γ -ray burst events ($\sim 30\text{--}40$ keV) and the fluences are slightly lower ($\sim 10^{-7}$ to $3 \times 10^{-6} \text{ erg cm}^{-2}$). Upon analysis of the burst energy versus time interval, no correlation was found between the energy emitted in the burst with the time either since the last burst or to the next burst. Furthermore, from the study of the number of observed bursts versus time

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interval, there is a tendency for the bursts to cluster on all timescales (no single burst is separated from the other bursts by more than a week²). Some of the primary characteristics which distinguish GB790107 from typical γ -ray sources are not unique to GB790107 since the other recurrent γ -ray burst sources (GB790305b and GB790324) have soft spectra.

Among the possible models which have been suggested for γ -ray bursters and which can be invoked to describe the characteristics of GB790107 are: localized thermonuclear runaways on the surface of an accreting neutron star^{7,8}, glitches⁹ and starquakes¹⁰, and episodic accretion associated with either disk instabilities¹¹ or comets¹². In the following we examine each model in turn and discuss its relevance to GB790107.

Models involving thermonuclear runaways, localized on the surface of a strongly magnetized neutron star, encounter severe difficulties in explaining GB790107, as the correlation between the energy of the burst with the recurrence timescale expected in this model is not observed. While, in principle, one could argue that the emitted radiation is strongly beamed, thus, masking an existing correlation, it seems that the observational data are sufficient to rule out such a relation. In addition, the rapid recurrence timescale of some of the soft γ -ray burst events ($\sim$ seconds) is also very difficult to explain in the context of the thermonuclear flash model. The highest mass accretion for which a thermonuclear instability is initiated is about $\dot{\Sigma}_{\max} \sim 10^{-11} M_{\odot} \text{ yr}^{-1} \text{ km}^{-2}$ (for otherwise the material will burn stably under nondegenerate conditions as would be the case for X-ray pulsars)^{4,5}. Since the minimum critical column density of matter for ignition is $\Sigma_{\min} \sim 10^{-16}$ to $10^{-15} M_{\odot} \text{ km}^{-2}$ the shortest possible recurrence timescale is

$$\tau_{\text{rec}} \approx 300 \left[\frac{\Sigma_{\min}}{10^{-16} M_{\odot} \text{ km}^{-2}} \right] \left[\frac{\dot{\Sigma}_{\max}}{10^{-11} M_{\odot} \text{ yr}^{-1} \text{ km}^{-2}} \right]^{-1} \text{ s} \quad (1)$$

This is in sharp contrast to the much shorter recurrence timescales observed occasionally in GB790107 as compared with those either observed or produced by theory for Type I X-ray bursters.

Another class of models that have been proposed involve glitches⁹ (and crustquakes¹⁰) in which readjustment of the neutron star interior (or crust) takes place. The main difficulty with the glitch model lies in the fact that no γ -ray bursts were observed to be associated with any glitch events from pulsars. (Pacini and Ruderman¹⁰ explain the absence of γ -ray bursts from the Crab and Vela pulsars by their distance.) On a theoretical basis, for those models where the glitches are due to the sudden unpinning of vortices from a pinned superfluid¹³ (with the coupling between the core superfluid and the outer crust taking place on a timescale of a second¹⁴), the glitch and post-glitch relaxation (both of which are determined by the interaction of vortices with nuclei in the crust) have timescales of ~ 20 to $10,000$ days¹⁵. It is thus unclear how such unpinning events could occur on the short timescales observed in GB790107. Alternatively, if we were to assume that the series of short timescale bursts represents not a series of unpinning events but some oscillations excited by one glitch or another type of disturbance (for example, a starquake associated with a phase transition¹⁶, transient accretion, nuclear burning^{17,18}) we are faced with the difficulty that such oscillations are short-lived¹⁸ (~ 1 s) and thus cannot explain the bursts observed at intervals of 100 to $1,000$ s.

Various instabilities in the accretion flow have also been suggested as mechanisms for the production of γ -ray bursts (as for example, may occur in the rapid X-ray burster MXB 1730–335). Among such mechanisms are a magnetic gating process¹⁹ and instabilities in the accretion disk¹¹. However, the basic difficulty with all of these models is that any relaxation oscillator that could apply to the rapid burster does not appear to apply to GB790107 because of the lack of correlation of energy with waiting time of the type observed for MXB 1730–335.

In contrast to the above models which are all plagued by severe difficulties, we suggest that a model involving the episodic accretion of comets from a comet cloud is consistent with the observational data (see refs 12, 20, 21). The main problem with this model, namely the retention of the comet cloud²², can be circumvented if the neutron star formed in a quiet collapse (without the ejection of a supernova shell). Note that the location of GB790107 (although admittedly uncertain²) suggests that the source lies close to the galactic plane and, thus, that the neutron star does not have a large velocity perpendicular to the plane. Loss of the comet cloud by neutron star recoil or by thermal ablation of the comets can be avoided²¹ even under less favourable neutron star formation (for example, white dwarf accretion from a low mass giant companion), provided that the comets are sufficiently massive $M_c \sim 10^{16}$ g.

The rate of capture of the comets from a comet cloud has been estimated to be as large as ~ 1 per year²¹ during comet showers, consistent with the observations of GB790107 considering the large uncertainties involved in the estimate. In this model the comets will hit the neutron star directly for periastron distances, b ,

$$b \leq 6 \times 10^6 \left[\frac{R_{\text{ns}}}{10^6 \text{ cm}} \right]^{4/3} \left[\frac{B}{10^{12} \text{ G}} \right]^{4/9} \left[\frac{\rho_c}{1 \text{ g cm}^{-3}} \right]^{-2/9} \times \left[\frac{r_c}{10^5 \text{ cm}} \right]^{-2/9} \left[\frac{M_{\text{ns}}}{1.4 M_{\odot}} \right]^{-1/9} \text{ cm} \quad (2)$$

where M_{ns} , R_{ns} and B are the mass and radius of the neutron star and the magnetic field strength respectively and where ρ_c and r_c are the comet density and radius. However, comets with $b < 3 \times 10^7$ cm (and possibly even larger) will be disrupted on their first passage only to be accreted on their second (or subsequent) passages²¹. Thus, most bursts in this model arise from disrupted comets, which is consistent with the fact that the bursts observed from GB790107 tend to appear in bunches.

Although we do not wish to examine in detail the question of γ -ray production we wish to make the following points in relation to our proposed model for GB790107. It has been recently argued²² that the accretion of comets by a neutron star will not lead to γ -ray bursts, but to a novel class of X-ray bursts. Indeed GB790107 may just represent this novel class since its spectrum is much softer (~ 30 – 40 keV) than that of typical γ -ray bursts. In addition, there is some theoretical evidence²³ which points to the importance of a strong field for confinement of the plasma to produce γ -rays from meteoritic impact. This is consistent with our requirement of a strong field to enhance the rate of capture via magnetic drag²⁴. Thus, implicit in our model is that the accretion takes place on a strongly magnetized neutron star.

If we adopt a fluence of 10^{-6} ergs cm^{-2} as a typical value for the γ -ray bursts, then the energy emitted is

$$E \sim 1.2 \times 10^{32} \left[\frac{d}{1 \text{ pc}} \right]^2 \text{ erg} \quad (3)$$

From the change of gravitational potential energy associated with the accretion of matter onto the neutron star surface, the energy released for a cometary mass, M_c , is

$$E \sim 1.86 \times 10^{35} \left[\frac{\epsilon}{0.1} \right] \left[\frac{M_{\text{ns}}}{1.4 M_{\odot}} \right] \times \left[\frac{M_c}{10^{16} \text{ g}} \right] \left[\frac{R_{\text{ns}}}{10^6 \text{ cm}} \right]^{-1} \text{ erg} \quad (4)$$

where ϵ is the efficiency of conversion of the gravitational energy into γ -rays. Taken together, equations (3) and (4) imply that the distance to the source is at most a few hundred pc for reasonable comet masses.

We remark that it has also been suggested²⁵ that γ -ray bursts could arise from the collision of neutron stars with planetesimals

formed within the accretion disk surrounding a neutron star, assumed to be in a close binary system with a low mass ($<0.05 M_{\odot}$) companion. This model avoids the problems associated with retaining the comet cloud by the neutron star. The major uncertainty with such a picture, however, lies in the fact that the main mechanism for producing the first generation of planetesimals with radii $\sim 10^5$ cm (suggested to operate in the solar-dust disk via a gravitational instability²⁶) will not operate under the influence of the strong tidal forces of the neutron star. It is thus not clear whether the growth time of the dust particles by sticking will be shorter than the orbital decay timescale of these particles.

As long as the neutron star can retain its comet cloud (for example, via a quiet collapse) the model can account for the frequency, clustering and random occurrence (both in terms of intervals between bursts and their strength) of the bursts observed from GB790107. Objections raised against this type of model in the past (that the radiation will not be emitted in γ -rays), in fact, favour this model because the spectrum is relatively soft. Furthermore, the model predicts that GB790107 is local and will undergo similar outbursts in the future.

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High-resolution electron microscopy of the high-temperature superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$

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The structure of the high-temperature superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$ has now been determined independently by neutron powder diffraction by several groups^{1–4}. These results all agree about the basic structure. It is an oxygen-deficient perovskite, with Ba and Y cations ordered over the A-sites of the $\text{A}_3\text{B}_2\text{O}_{9-\delta}$ structure, in the sequence Ba–Ba–Y. The two oxygen vacancies ($\delta = 2$) are perfectly ordered. There are no oxygen atoms on the Y-planes, and the oxygen vacancies on the Cu-planes, between the two Ba-planes,

leave large tunnels along the (010) direction. This is only the average structure, as it was determined by a diffraction technique; it is useful to verify by electron microscopy the local degree of order and crystal perfection. In particular, electron microscopy will reveal the existence of any very long-range superstructures, or of isolated imperfections, such as interstitial planes of Ba and/or Y atoms, or shear planes. Here we report high-resolution electron microscopic observations of the $\text{YBa}_2\text{Cu}_3\text{O}_7$ crystals used in the neutron powder determination of the structure by Capponi *et al.*¹. The crystals are seen to be highly twinned on the (110) planes, but are very well ordered. The Ba–Ba–Y ordering of planes along the *c*-axis is perfect; no interstitial Ba and Y planes, or shear planes, were seen. Simulated images, using the structural data of ref. 1, are shown to be in good agreement with observed images.

The crystal powder was prepared for electron microscopy by the standard technique for oxide powders. A suspension of the crystals in dichloromethane was ground in an agate pestle and mortar. Crystals were recovered from the suspension on a porous carbon film. Observations were performed at 200 kV on a JEOL-200CX microscope, the objective lens spherical aberrative constant, $C_s = 1.05$ mm, equipped with a LaB₆ filament and a top-entry tilting stage ($\pm 7^\circ$).

Simulations of electron microscope images were performed on a VAX-780 computer using the programs SHRLI⁵. These programs are based on the multi-slice theory of ref. 6. The simulated images were displayed on a Ramtek raster graphics terminal.

Crystallites were observed to lie on the carbon support film in all possible orientations, suggesting that they do not have a strongly preferred cleavage plane. The crystallites were highly twinned on (110) planes, and showed evidence for the existence

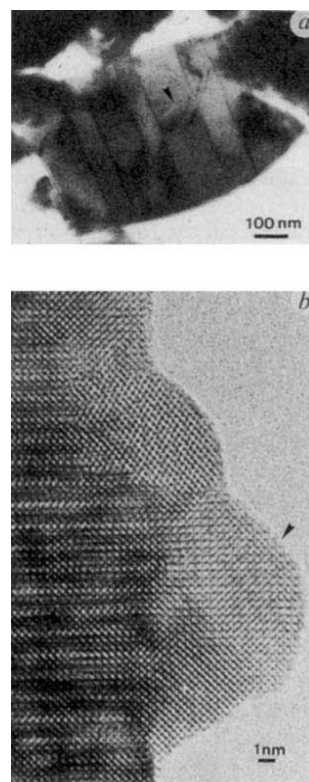


Fig. 1 *a*, Bright-field image of a crystallite of $\text{YBa}_2\text{Cu}_3\text{O}_7$, showing twinning along (110) planes. There is evidence for a non-planar interface (see arrow). *b*, High-resolution electron micrograph of $\text{YBa}_2\text{Cu}_3\text{O}_7$ crystallite in the (010) orientation; defocus = -50 nm. The crystal is recrystallizing at its surface, where new phases appear. The recrystallized region (arrowed) has a lattice parameter $\sim 7\%$ larger than the *a*-spacing of $\text{YBa}_2\text{Cu}_3\text{O}_7$.

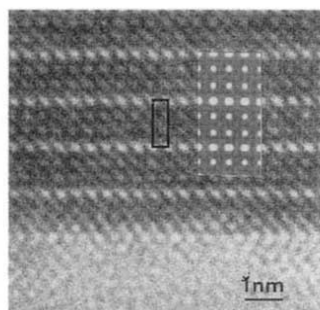
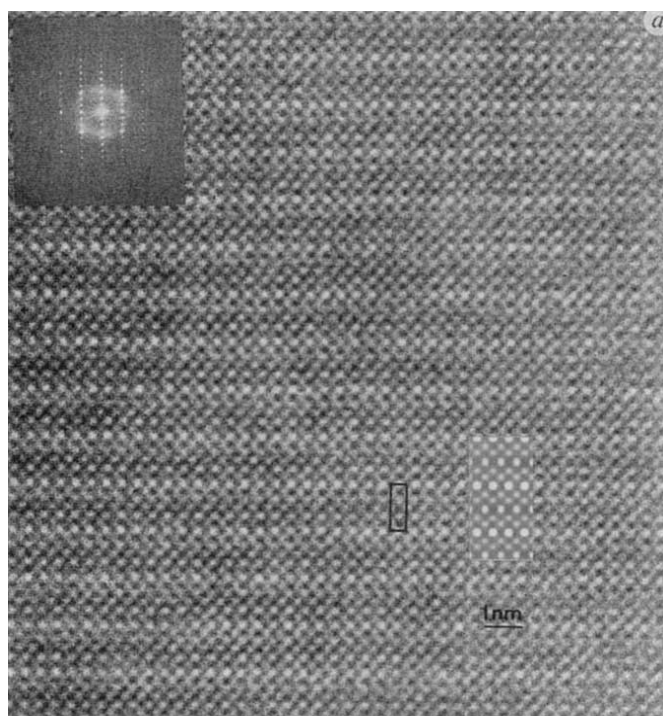


Fig. 2 *a*, High-resolution electron micrograph of $\text{YBa}_2\text{Cu}_3\text{O}_7$ in the (010) (or possibly (100)) orientation, and corresponding optical diffraction pattern (top inset). The (010) simulated image for a crystal thickness of 3.1 nm and a defocus of -50 nm (lower inset) is in agreement with this image, and resembles the projected potential distribution. The heavy atoms are darker. *b*, High-resolution electron micrograph of $\text{YBa}_2\text{Cu}_3\text{O}_7$ in the (010) orientation, and corresponding simulated image for a crystal thickness of 5.7 nm and defocus of -70 nm. This image is the near-focus maximum-contrast image.

of non-planar types of interface (see Fig. 1*a*). They were also fairly sensitive to beam heating. The orthorhombic to tetragonal phase transition was observed in the microscope on intense beam heating⁷. Some crystals were observed to undergo recrystallization into different phases in the microscope (see Fig. 1*a*), but with a little care not to overheat the crystals, it was possible to observe them in their original state.

Images of crystals in the (010) orientation showed the crystals to be well ordered, with no superstructure or interstitial Ba or Y planes. Simulated electron microscope images are in good agreement with the observed images for the (010) orientation. Figure 2*a* corresponds to the image simulated for a defocus of -50 nm (see inset); this is the defocus which gives an image most like the projected crystal potential (see Fig. 3). The row of brighter spots in the (010) orientation corresponds to the tunnels left by the ordered oxygen vacancies; note that the

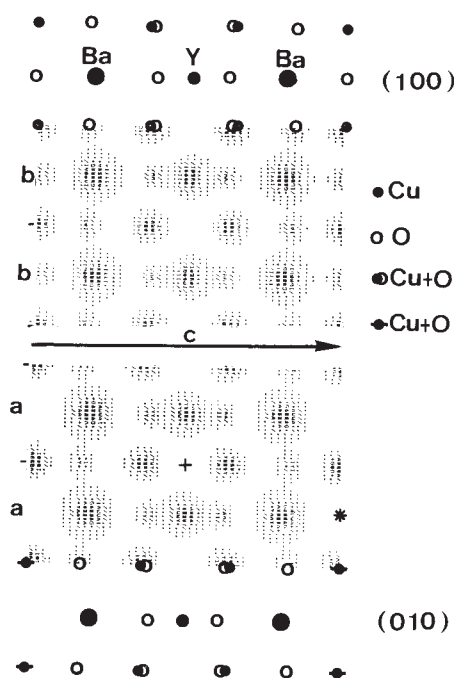


Fig. 3 Projected potential for $\text{YBa}_2\text{Cu}_3\text{O}_7$ in the (100) and (010) orientations as seen by 200-kV electrons. Two unit cells are shown. Note that the oxygen vacancy tunnels are larger on the copper planes (*) than on the yttrium planes (+). Lattice dimensions: $a = 3.8206$ Å; $b = 3.8851$ Å; $c = 11.6757$ Å.

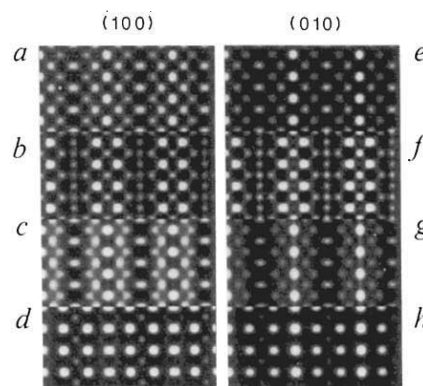


Fig. 4 Simulated 200-kV electron micrographs for $\text{YBa}_2\text{Cu}_3\text{O}_7$, based on the structural data of ref. 1. *a-d* are for the (100) orientation and *e-h* are for the (010) orientation. The brighter spots in *a* and *e* correspond to oxygen atom positions, and the oxygen vacancy tunnel positions, respectively. Note that the bright spots in *d* and *h* are on the copper atom positions. Orientation, crystal thickness (nm) and defocus (nm) are as follows: *a*, (100), 3.1, -50 ; *b*, (100), 3.1, -70 ; *c*, (100), 5.7, -45 ; *d*, (100), 5.7, -70 ; *e*, (010), 3.1, -50 ; *f*, (010), 3.1, -70 ; *g*, (010), 5.7, -45 ; *h*, (010), 5.7, -70 . A defocus of -50 nm corresponds to the image of the projected potential, -70 nm to the maximum-contrast image near focus.

tunnels on the Cu-plane are larger than those on the Y-plane. Figure 2*b* shows the experimental and simulated images for a slightly thicker region of the same crystal and a defocus of -70 nm. This is the 'near focus' image which has maximum contrast, and is invariant for a large defocus range of ~ 15 nm. It will be noticed that the line of bright spots is still on the same oxygen-deficient plane, but that the bright spots now lie on the Cu atom positions. This image is therefore not a simple projection of the structure.

It can be seen from the image simulations for the (010) and (100) orientations (Fig. 4) that in general the difference is not very marked. This is not surprising, since the major difference

is in the position of one oxygen atom. However, the difference for $t \approx 6$ nm and a defocus of -70 nm, the maximum contrast image, is certainly sufficient to be detected experimentally.

Our images show the $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ crystals to be well ordered, and simulated electron microscope images, using the structural data from ref. 1, are in good agreement with our observations. Neutron measurements which are more sensitive to the average oxygen structure and stoichiometry than electron microscopy, indicate that the oxygen defect concentration must be small, and that the formula is $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ where $x < 0.1$.

Less well-ordered specimens, with a high defect concentration, have been observed⁸, with widespread extrinsic planar defects. As the single-phase $\text{YBa}_2\text{Cu}_3\text{O}_7$ crystals that we used have been shown to have a sharp onset of superconductivity at 100 K ¹, we conclude that the extrinsic planar defects do not play a positive role in the superconducting phenomenon.

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The room-temperature structure of the $\sim 90\text{-K}$ superconducting phase $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$

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Like many others^{1,2} we have explored phase relationships in the Y-Ba-Cu-O system, isolated the pure ($>98\%$ by X-ray diffraction) phase $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$, measured its electrical properties, and examined it by high-resolution electron microscopy. Here we report on the room-temperature structure of the superconducting phase of this system. The particular sample of $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ that was studied had been prepared by the direct reaction of stoichiometric amounts of Y_2O_3 (99.99%), CuO (99.999%) and BaCO_3 (99.999%), thoroughly mixed, pressed into a pellet and heated in air at 925°C in an alumina crucible for 16 h. The sintered specimen was then reground, pressed into a pellet and reheated at 925°C in O_2 for five days, followed by annealing at 500°C in O_2 for 48 h. An X-ray powder diffraction pattern obtained with a Guinier-Hägg camera using monochromated $\text{CuK}\alpha_1$ radiation gave refined orthorhombic cell parameters for the specimen of $a = 3.820(1)\text{ \AA}$, $b = 3.886(1)\text{ \AA}$ and $c = 11.703(1)\text{ \AA}$. (The oxygen content is not known but, for our method of preparation, is generally reputed to be $7-x \approx 6.9$.)

The freshly prepared pure compound had the resistance-temperature relationship shown in Fig. 1a, with a midpoint ' T_c ' of $90.5 \pm 1.0\text{ K}$ and a transition temperature range (10-90%) of 2.0 K . The a.c. magnetic susceptibility of the sample, measured at a frequency of 187 Hz , is shown in Fig. 1b. A negative step as a function of decreasing temperature is seen at the transition point demonstrating the existence of a Meissner effect. A recent description of work at AT&T Bell Laboratories³ and Arizona

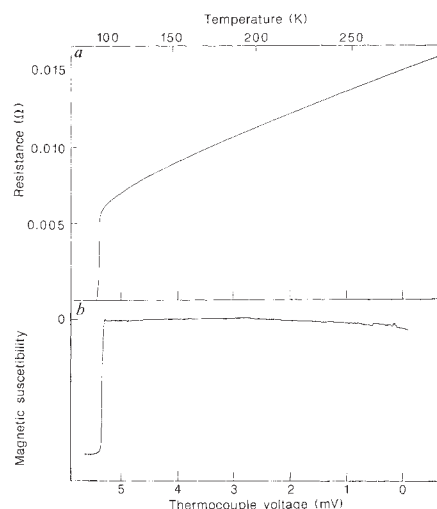


Fig. 1 *a*, The resistance of the sample as a function of temperature. The horizontal scale is the copper-constantan thermocouple voltage with its reference junction at the ice point. The corresponding temperatures are indicated on the upper scale. The room-temperature resistivity of the sample is $13 \pm 5\text{ }\mu\Omega\text{m}$, the large uncertainty being due to the irregular shape. *b*, The a.c. magnetic susceptibility of the sample measured at a frequency of 187 Hz as a function of temperature. The origin of the susceptibility is shown and the units are arbitrary.

State University showed high-resolution images of a superconducting $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ sample that was characterized by numerous defects. In contrast, we observed no defects in any of our samples when transferred directly from furnace to electron microscope. Figure 2 shows a high-resolution lattice image, typical of the many crystals of our superconducting sample that we examined, along with its corresponding selected area diffraction pattern (SADP). The simplicity of the SADP is matched by the regularity of the corresponding lattice image. Clearly, our freshly prepared material is essentially perfectly periodic.

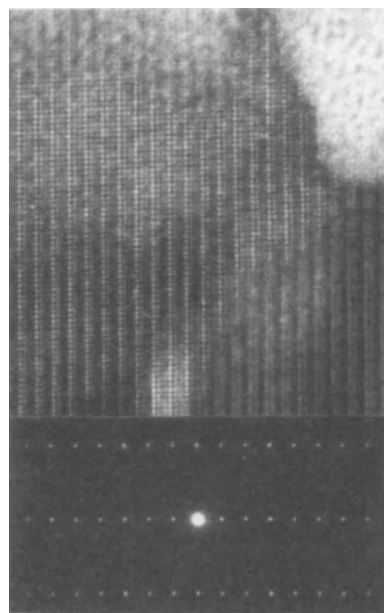


Fig. 2 Lattice image typical of freshly prepared $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ zone axis $[100]$; c -axis is horizontal, b -axis is vertical. The perovskite subcell and $3 \times c$ 'superstructure' are obvious, as is the high degree of perfection characteristic of such specimens. The corresponding orientated diffraction pattern is inset (JEOL 200CX microscope).

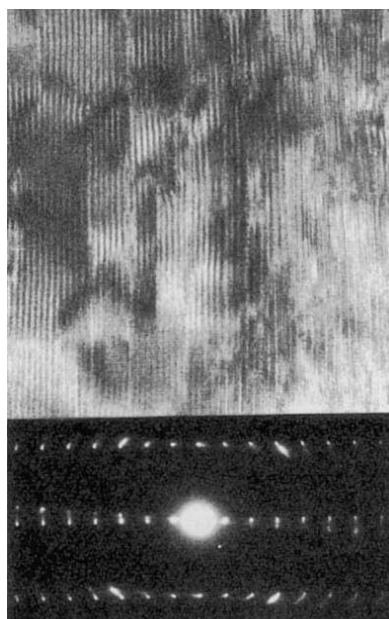


Fig. 3 Bright-field image orientated as in Fig. 2 of a $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ specimen that has been exposed to the atmosphere for several days. Note the intense disorder and disruption of the parent structure. The corresponding orientated diffraction pattern (inset) shows streaking along c^* and rotation about the projection axis.

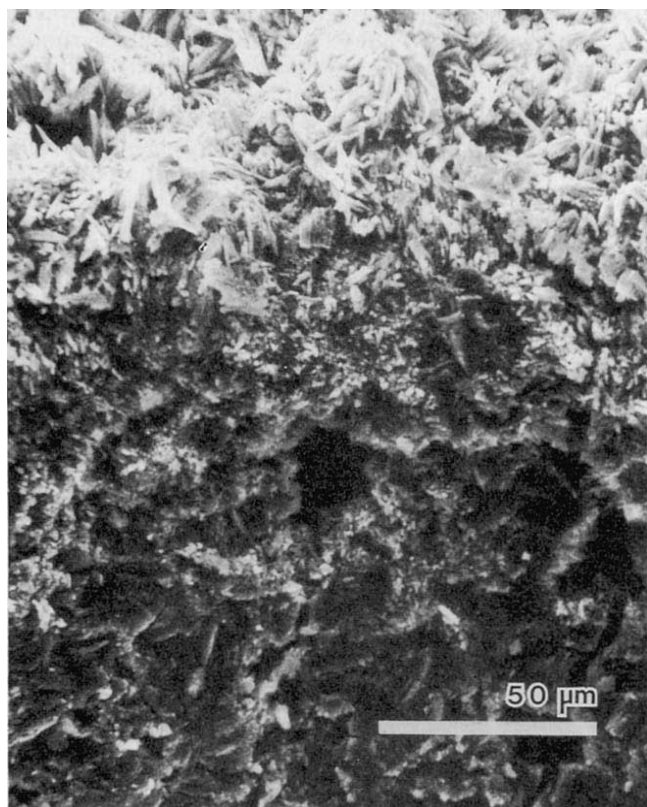


Fig. 4 Scanning electron micrograph (secondary electron image) at a fracture surface through a cold-pressed, sintered pellet of $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$ after accelerated atmospheric corrosion. The upper part of the micrograph exhibits the exposed surface of the pellet with micrometer-sized blade-like BaCO_3 crystals. The lower part shows the relatively uncorroded fracture surface with fissures and pores.

This result conflicts with the speculation³ that the defects depicted there might be responsible for the superconductivity.

But we have also observed that the pure material deteriorates when exposed to the atmosphere, and that its degradation is manifested in two stages. In stage 1 defects appear in diffraction-contrast images, including planar faults parallel to (001) which, at higher magnifications, resemble some of those in the higher-resolution images in ref. 3. In our samples defect densities vary somewhat from grain to grain but, in general, fault density and depth of penetration into the crystals increase with further exposure. As shown in Fig. 3, this atmospherically degraded material gives rise to imperfect SADPs and lattice images. The streaking present along c^* in the SADP is matched in the lattice image by the planar defects parallel to (001), while the rotation about the projection axis corresponds to the waviness of the lattice fringes 'perpendicular' to c^* .

Stage 2 is the bulk decomposition of the 90-K superconducting phase. Products of its decomposition in air have been identified by X-ray powder diffraction as $\text{Ba}_2\text{Cu}(\text{OH})_6$, BaCO_3 , CuO and $\text{Y}(\text{OH})_3$. Studies of enhanced degradation in air at 40 °C saturated with water vapour have shown that bulk decomposition begins at the surface, from which microcrystals of BaCO_3 grow. Figure 4 shows a scanning electron microscope image of the surface of the degraded specimen.

Our measurements and qualitative observations demonstrate that the presence of defects is not a pre-requisite for 'high-temperature' superconductivity in this new class of superconducting materials, and suggest that at least some of the observed defects are characteristic of the decomposition of the superconducting phase, rather than of the pure material itself.

Superconductivity may still be observed, even in the partly degraded material, as long as continuous electrical pathways exist. But the exclusion of water vapour is clearly of prime importance in any consideration of potential applications of this high-temperature superconductor.

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High electrical conductivity in doped polyacetylene

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The electrical conductivity of conducting polymers results from mobile charge carriers introduced into the π -electron system through doping^{1,2}. Because of the large intra-chain transfer integrals, the transport of charge is believed to be principally along the conjugated chains, with inter-chain hopping as a necessary secondary step. In conducting polymers, as in all metals and semiconductors, charge transport is limited by a combination of intrinsic electron-photon scattering and sample imperfection. Although relatively high conductivities ($\sigma \approx 1,000 \text{ S cm}^{-1}$) have been reported for partially orientated and heavily doped polyacetylene¹⁻³, the absence of a metal-like temperature dependence implies that the observed values are not intrinsic. In doped polyacetylene,

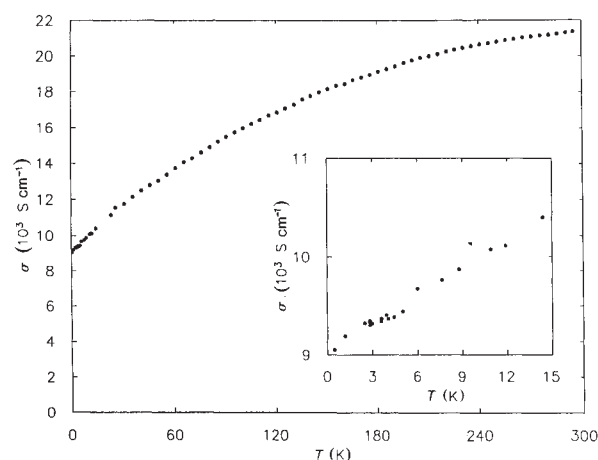


Fig. 1 $\sigma_{\parallel}(T)$ for a fourfold stretched sample at 10 kbar; the inset shows the temperature dependence below 4 K in more detail. At room temperature, the conductivity is greater than $20,000 \text{ S cm}^{-1}$; at 0.5 K the conductivity is still above $9,000 \text{ S cm}^{-1}$.

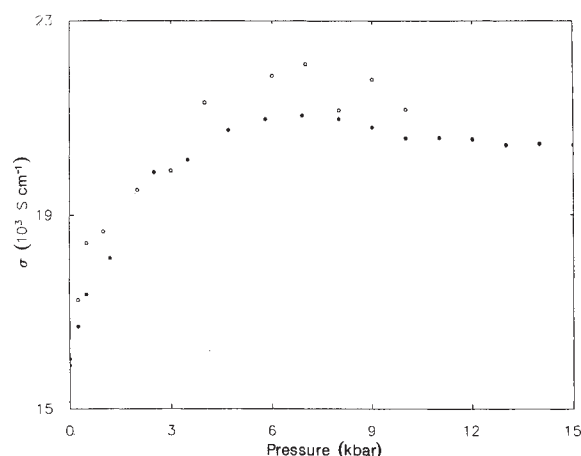


Fig. 2 Pressure dependence of $\sigma_{\parallel}$ at room temperature from 1 atm to 15 kbar. Solid dots and open circles represent two independent samples with stretching ratios of 6:1.

$(\text{CH})_x$, electrical transport can be limited both by microscopic defects (leading to scattering and localization) and by the more macroscopic complex fibrillar morphology¹² and associated interfibrillar contacts. Thus, with improvements in material quality, one might anticipate corresponding improvements in the electrical conductivity. Here we report the synthesis of polyacetylene with fewer sp^3 defects than in material prepared by other methods. The higher-quality material exhibits substantially higher electrical conductivity; maximum values of $>20,000 \text{ S cm}^{-1}$ are obtained after doping with iodine. The conductivity has been measured as a function of temperature and pressure: at 0.48 K and 10 kbar, iodine-doped samples remain highly conducting ($\sigma \approx 9,000 \text{ S cm}^{-1}$).

Free-standing polyacetylene films (thickness $\sim 30 \mu\text{m}$) were synthesized using the Zeigler-Natta catalyst technique, initially developed by Shirakawa and colleagues⁴, with important modifications introduced by Naarmann⁵. The catalyst (tetrabutoxytitanium, 31 ml, and triethylaluminium, 41 ml, suspended in silicone oil, 50 ml) was stirred for two hours at 120°C , followed by stirring and degassing (pumping) while cooling slowly to room temperature. The polymerization reaction was carried out at room temperature (rather than using⁴ toluene with polymerization at -78°C) in a controlled atmosphere drybox, followed by washing to remove all the catalyst (3 hours in toluene followed by 16 hours in methanol containing 6% HCl, and finally two periods of 1–1.5 hours each with methanol). The resulting films are roughly a 50/50 mixture of *cis*- and *trans*-polyacetylene; elemental analysis: C, 91.7–92.2%; H, 7.6–7.7%; O, $<0.5\%$; Al/Ti, 0.01%. Isomerization to the all-*trans* material can be achieved by subsequent heating or by doping. The material can be stretch-oriented with maximum elongation ratio of about 6:1.

The polymer synthesized by this technique has been thoroughly characterized⁵. In most respects it is essentially identical to polyacetylene prepared by the Shirakawa technique. The material has the characteristic fibrillar morphology (approximately the same fibril diameter as Shirakawa material) with a density of 0.5 g cm^{-3} . X-ray scattering studies show features which are indistinguishable from Shirakawa material. The principal difference is a major reduction in the number of sp^3 defects to a level which is not detectable by high-resolution ^{13}C nuclear magnetic resonance. No bands corresponding to $-\text{CH}_3-$ or $-\text{CH}_2-$ could be detected at wavelengths of 2,960, 2,910 and $2,830 \mu\text{m}$ in the infrared spectrum. The low density of sp^3 defects implies a higher degree of chain perfection in this material.

The $(\text{CH})_x$ films were doped to saturation by immersion for one hour in a saturated solution of iodine in CCl_4 , followed by rinsing three times in pure solvent for two minutes each. Since

$(\text{CCl}_3)^-$ is produced in the photon catalysed reaction, $2\text{I}_2 + \text{CCl}_4 \rightarrow (\text{CCl}_3)^- + \text{ICl}$, and attacks the double bonds on the polyacetylene chain, care must be taken not to expose the solution to light. Weight uptake analysis indicates a doping concentration of $y \approx 0.06$ assuming a composition of $[(\text{CH})^{+y}(\text{I}_3^-)_y]_x$; we find that most of the doping occurs during the first 15 minutes of immersion. Immersion in the iodine solution for more than 70–80 minutes yields films with lower conductivity, possibly due to degradation of the polymer.

Immediately after rinsing, four copper wires were attached to the doped film (under an inert atmosphere) using electrodag. The samples, mounted with the stretch direction either parallel ($\sigma_{\parallel}$) or perpendicular ($\sigma_{\perp}$) to the current flow, were placed in a teflon cell for high pressure measurements (1 atm to 15 kbar). The pressure cell was designed for interchangeable use in a closed cycle Displex refrigerator for measurements from 20 K to 300 K and in a ^3He cryostat for measurements down to 0.48 K.

The most striking feature of the Naarmann polyacetylene is the high electrical conductivity. Figure 1 shows $\sigma_{\parallel}$ as a function of temperature for a fourfold stretched sample at 10 kbar. At room temperature, the conductivity is greater than $20,000 \text{ S cm}^{-1}$; at 0.5 K the conductivity is still above $9,000 \text{ S cm}^{-1}$. The inset shows the temperature dependence below 4 K in more detail. The results shown in Fig. 1 are typical, with good reproducibility from sample to sample. For a single sample, the conductivity was observed to decrease monotonically

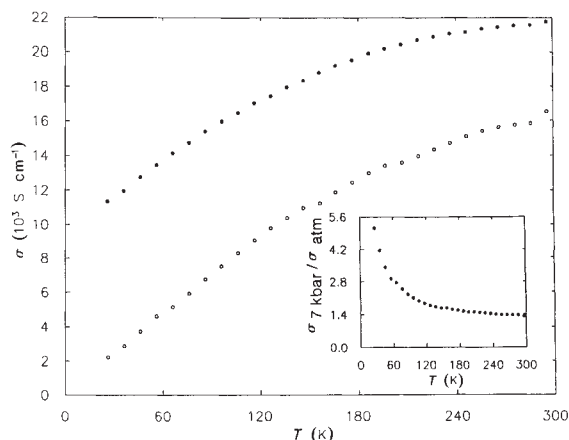


Fig. 3 Temperature dependence of $\sigma_{\parallel}$ at 7 kbar (solid dots) and at 1 atm (open circles); stretching ratio 6:1. Pressure suppresses the decrease in $\sigma_{\parallel}(T)$ at low temperatures. Inset: $[\sigma_{\parallel}(7 \text{ kbar})/\sigma_{\parallel}(1 \text{ atm})]$ as a function of temperature.

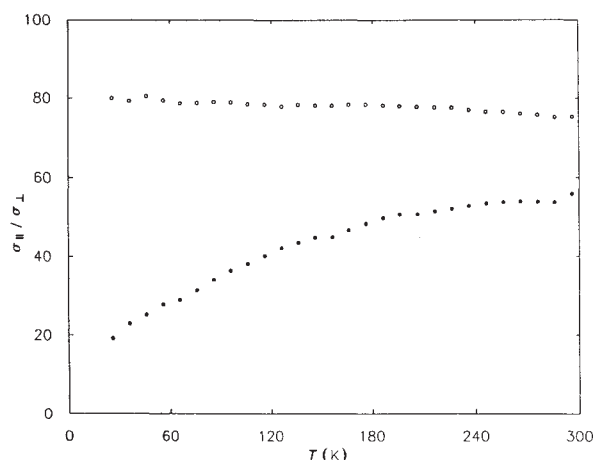


Fig. 4 Temperature dependence of $(\sigma_{\parallel}/\sigma_{\perp})$ for pressures of 1 atm (solid dots) and 7 kbar (open circles); stretching ratio 6:1.

cally with time (down by about 30% over a period of a week) indicative of the irreversible degradation known for iodine doping^{1,2}.

Figure 2 shows the pressure dependence of $\sigma_{\parallel}$ at room temperature from 1 atm to 10 kbar. The parallel conductivity has a gentle maximum at about 7 kbar; the data are reversible on cycling up to 15 kbar. The perpendicular conductivity (not shown) increases by about 10% below 1 kbar and remains constant (within measurement error) at higher pressures. The pressure dependence of $\sigma_{\parallel}$ and $\sigma_{\perp}$ was checked on a number of different samples and found to be characteristic.

Figure 3 shows the temperature dependence of $\sigma_{\parallel}$ at several different pressures; high pressure suppresses the decrease in $\sigma_{\perp}(T)$ at low temperatures. This effect is emphasized in the inset to Fig. 3 where we plot the ratio $[\sigma_{\parallel}(7 \text{ kbar})/\sigma_{\parallel}(1 \text{ atm})]$ as a function of temperature.

The anisotropy of the conductivity is relatively large compared to that obtained from previous measurements on material prepared by the Shirakawa method; we find $(\sigma_{\parallel}/\sigma_{\perp}) \approx 80$ at 7 kbar. The anisotropy is somewhat higher at high pressure, as $\sigma_{\parallel}$ increases with pressure while $\sigma_{\perp}$ remains essentially constant. The temperature dependence of $(\sigma_{\parallel}/\sigma_{\perp})$ is shown in Fig. 4 for pressures of 1 atm and 7 kbar. Note that at high pressure, the ratio is constant.

The observation of a temperature- and pressure-dependent anisotropy suggests that the transport data are beginning to provide information on intrinsic processes. The magnitude and temperature independence of $\sigma_{\parallel}(T)$ below 1 K implies genuine metallic behaviour for heavily doped polyacetylene (consistent with specific heat⁶, thermopower⁷ and susceptibility data⁸) and rules out transport by hopping among strongly localized states. Note, however, that $\sigma_{\parallel}(T)$ increases with increasing temperature implying that phonon-assisted transport (either through microscopic localized states or across inter-fibrillar barriers) is involved. Thus, although this modified synthesis yields significantly higher-quality polyacetylene (with $\sigma_{\parallel}$ within about a factor of 20 of the conductivity of copper) the transport is still limited by material imperfection.

Although improvement of solid-state properties through higher-quality materials is a general feature of materials science, there has been little optimism that this rule would be applicable to conducting polymers. Perhaps the reason for this was the argument that the high level of impurities in doped conducting polymers would negate any improvements towards molecular chain perfection. The present study demonstrates that this is not the case in polyacetylene or (by implication) in other conducting polymers: the achievement of still higher-quality material from improved synthesis and processing can be expected to lead to correspondingly better electronic properties.

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Note added in proof: H.N. recently announced further improvements in the preparation of oriented polyacetylene leading to a room temperature electrical conductivity of $\sim 1.5 \times 10^{+5} \text{ S cm}^{-1}$ with an anisotropy of $\sim 10^3$ (*American Chemical Society Meeting Symposium on Conducting Polymers, Their Emergence and Future*; April 7-8, 1987, Denver, Colorado).

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Lightning triggered from the Earth's magnetosphere as the source of synchronized whistlers

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Specific patterns established by natural radio signals echoing periodically in a magnetospheric whistler duct¹⁻³ are sometimes observed to indicate strongly preferred times at which identical, well-defined whistlers occur. Ordinarily such whistlers would be assumed to originate in the stronger ground strokes⁴ of spontaneous lightning. These nonrandom whistlers are therefore unexpected and raise the possibility that some whistler-source discharges may be triggered from the magnetosphere. The same possibility could also be predicted independently from another type of our recent observations. Selected examples of nonrandom whistlers are given here and the supporting observations described. In the absence of a previously developed theoretical explanation, a trigger mechanism is outlined which emphasizes discontinuous discharge to the upper atmosphere⁵. Such a mechanism might help explain several related phenomena.

Examples to be shown are from Southern Hemisphere data recorded at Siple Station, Antarctica, 76° S, 84° W geographic. Two nearly identical whistlers W_1 and W_2 occur in Fig. 1, each arriving about two seconds after its Northern Hemisphere source discharge. The second (W_2) is approximately in phase with echoes due to the first (W_1). If many whistlers were found with phasing identical to W_2 , then their source discharges could no longer be thought of as spontaneous. Here, solid diagonal lines repeat a base period measured near 3.0 kHz between the leading edges of W_1 and its 'two-hop echo', e_1 .

Because they are quickly eroded by path-propagation losses, successive echoes e_1 - e_5 weaken and collapse toward a single frequency. This illustrates two important points: first, if periodic signals are to continue echoing in a long train, supplemental energy usually is required and second, if a unique period is maintained, it is usually because of a dominant signal echoing at a single frequency. Here 'echo' E_2 shows supplemental energy mostly at constant frequency. The starting time of E_2 shows that the leading edge of W_1 has established the periodicity.

With energy now being added, each echo grows gradually and becomes more complex until E_5 (not labelled) and supplemental emission T_2 result. W_2 appears to follow precisely in phase with T_2 , so a Northern Hemisphere source discharge might be triggered in connection with T_2 's northern reflection.

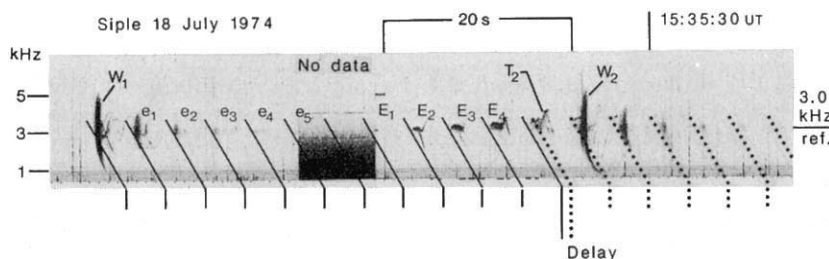


Fig. 1 A sequence of echoes (e_1 – e_5) started by a previous whistler (W_1), later shows supplemental energy in the form of emissions E_1 – E_5 (E_5 not labelled) and T_2 . A whistler (W_2) then follows approximately in phase. Solid and dotted lines indicate uniform periodicity, measured at about 3.0 kHz. Delay is discussed in the text. Note how W_2 'falls on top of' the weak emission E_6 (not labelled). This specific configuration, whistler 'on top of' an emission, has characterized probable triggered whistlers like W_2 in about ten other echo sequences found to date. An example appears as Fig. 1 of ref. 1.

However, in the cases studied to date, whistlers like W_2 are more consistently associated with stimulation and more gradual (therefore more delayed) growth of signal energy, such as that supplementing the echoes at nearly constant frequency. With respect to the start of E_5 , delay of about 27% of the base period near 3.0 kHz is passed on to W_2 and its echoes, which then maintain the original periodicity (dotted lines). Such delay is probably due to several aspects of the triggering process.

Two other examples (not shown) of whistlers in patterns identical to that of Fig. 1 were recorded on 21 March 1977 at Roberval, Quebec, Canada near the northern end of the Siple geomagnetic field line. These examples are significant because they show the whistler source-stroke sferics (radio impulses). These are prominent, but not otherwise extraordinary, for each whistler, whether spontaneous or triggered.

Nonrandom whistlers also occur in less transient, more steady-state situations. For example, with supplemental energy apparently drawn from a band of hiss (Fig. 2), uniform echoing remains evident during trains which are longer and less dependent on regeneration (extension) by any single synchronized whistler. Such whistlers are seen to be numerous and very similar however, probably because long echo trains provide more opportunity for regular occurrence and for repeated identification. As the first 'echoes' become established, their separation may increase by as much as 10% to match that of the synchronous whistlers. Compared to the first example given, both stimulation of echoes from hiss and triggering of whistlers appear to be more continuous and simultaneously under periodic control which is quite stable. The six synchronized whistlers in Fig. 2 are spread over an unusually long train of 59 echoes. Two different whistlers (W_3 and W_7) have identical echoes separated by $N=37$ intervals, so the average interval can be measured with $\pm 0.3\%$ accuracy. It is within 4% of the basis of spacing of all the whistlers and identically equal to the basis of spacing of the whistlers W_3 , W_5 and W_7 which are within $\pm 0.25\%$ of perfect periodicity. Note that anomalous W_6 timing is to be expected because W_5 has no opportunity to echo. Taking the worst-case irregularity of the period to be equivalent to the poor (phase) definition of individual echoes, a simple estimate gives a maximum probability of 1/15,625 that all six whistlers are independent random events.

Other observations independently suggest the existence of discharges triggered from the magnetosphere. First, a type of radio reflection change⁶ thought to register a perturbation of electron density at ionospheric D-region altitudes, sometimes occurs very impulsively in about 50 ms^{7,8}, possibly indicating charge redistribution at ten or more times the normal rate. Since this rise time is about that of transient ionization columns detected by radar⁹, both types of event might involve similarly impulsive currents enduring about as long as some lightning flashes¹⁰. Lightning (predominantly simultaneous) has been clearly indicated with each of about 25 impulsive reflection change events studied to date.

In a second case, significant, impulsive reflection changes (with sferics) occurred selectively at the times of energetic, radiation-belt electron bursts detected independently during precipitation into the ionosphere¹¹. Impulsive changes were observed only with some of the bursts, so it seems likely that

an impulsive current or other possible cause⁴³, perhaps including simultaneous lightning, was triggered by particular bursts.

Third, these same electron bursts were repeatedly correlated with whistlers and characteristic emissions¹¹ indicating pitch-angle scattering of magnetospheric electrons as the source of a burst^{12,13}. Furthermore, the emissions were a type previously indicative of bremsstrahlung X-ray bursts energetic enough to be detected at 30-km (7 g cm^{-2}) balloon altitude¹⁴. If we could show that such X-ray bursts sometimes trigger discharges which produce new whistlers, then we would predict the occurrence of nonrandom whistlers similar to the examples given here. We now discuss this possibility.

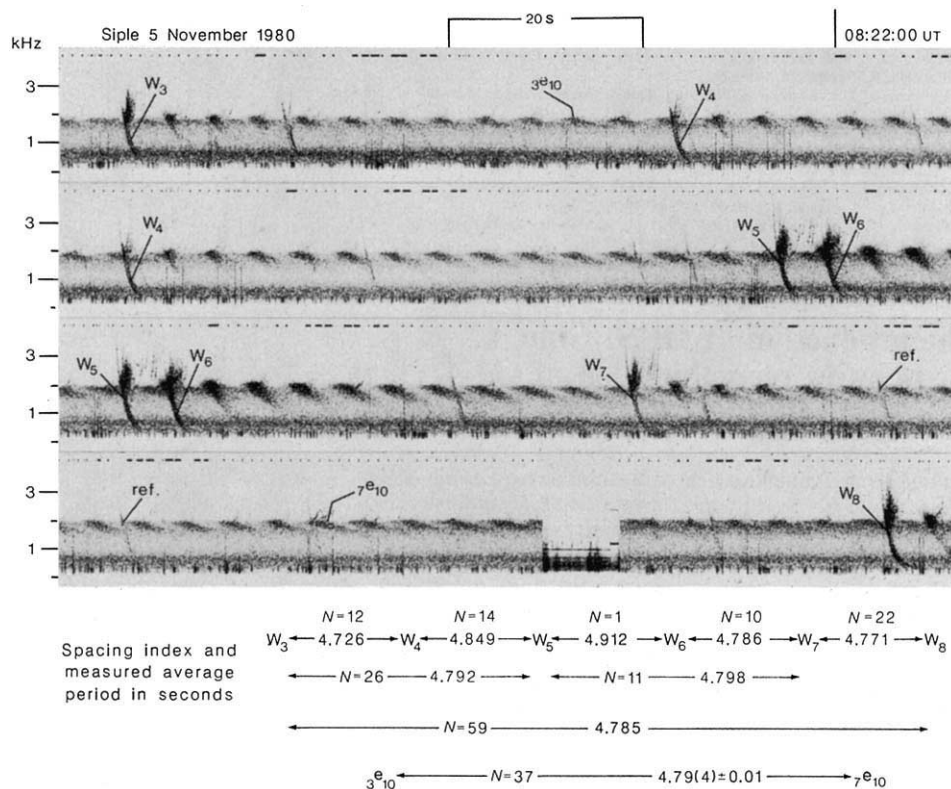
With other analyses or reports^{15–25} of similarly impulsive and/or synchronized behaviour, the evidence given above suggests a common mechanism as follows. On rare occasions, charge lowered to earth by a spontaneous lightning flash is immediately replaced by an impulsive current which taps a source sometimes as high as ionospheric altitude. Conversely, sometimes a stroke to ground immediately removes charge lowered by a spontaneous impulsive current which partially neutralizes the top of a tropospheric charge dipole. Such currents probably require temporary 'filaments' of enhanced conductivity to be formed in advance. Conditions affecting filament formation, particularly their length, continuity, and number determine which macroscopic phenomena occur. Energy is supplied primarily from the charge dipole.

One type of conductive filament which could actively conform to a path of minimum potential difference might be formed by a string of 'bubbles' even if these carried no net electrical charge²⁶. Formation might begin when a physical 'phase change' initiates local 'electrical boiling'^{27,28} in the lower atmosphere. Just as ordinary boiling indicates heat flow greater than free or organized^{29,30} convection can support, the transition to electrical boiling would mean that electrostatic 'leakage' currents are otherwise unable to transfer charge at the higher net rate required by other processes. On a smaller scale, the transition to a (very stable) organized³⁰ state similar to a string of (higher altitude) 'bubbles' might be said to occur when increasing voltage produces luminous structures in a low-pressure laboratory gas-discharge tube. Analogously, we are discussing a physical regime which temporarily produces a steady-state charge distribution having no time to relax, in the atmospheric example, to that of an equilibrium electrosphere³¹.

An assumption of local electrical phase change means that electrostatic (equilibrium) computational models can be expected to approximate correctly only those (steady-state) parts of the actual behaviour which remain nearly electrostatic. The following preliminary analysis is therefore somewhat fragmentary, perhaps like a series of snapshots.

Starting with 3,000 bubbles $10 \times 10 \times 10\text{ m}^3$ formed at 1 bubble per second and spaced 10 m apart, we find that it takes 100 min to fill a 60-km column. Assume a field 0.3 kV m^{-1} (breakdown) at 70 km and a spherical bubble of radius r carrying (negative) $1/25\text{ mC}$ (downward). This gives 0.12 C on the filament. If viscous drag is to hold the (formation) velocity to 10 ms^{-1} , then by Stokes Law, the radius r is about 4 m. Note that $1/25\text{ mC}$ on a 600-m^2 bubble produces 7.5 kV m^{-1} , breakdown at about 42 km. Above that, such (transient) bubbles are 'overcharged'.

Fig. 2 Whistler 'echoes' form long trains apparently by drawing energy from a band of hiss. Each panel overlaps the one below it and some whistlers are shown twice for continuity. Whistlers and echoes have the same average periodicity throughout. Typical measurement accuracy of a whistler's position is ± 12 ms, made possible by some well-defined whistler segments. These whistlers travelled a path intersecting the Earth's surface in central Quebec, Canada at about 53° N geographic. A high-latitude winter storm is therefore implied, for which a model study finds coupling to the ionosphere possibly more significant⁴¹. Several weaker whistlers having accurate $N+1/2$ phases could be due to a different kind of triggering. An event of this clarity, having just enough whistlers to make synchronization obvious, probably requires unusual matching between magnetospheric echoing and (cloud) charge-separating conditions. We estimate (very roughly) five or fewer such events a year worldwide.



We will assume the inconsistency means that the region 40–70 km is actually one of forced, electrical convection irregularities³² or vortices and scale-size reduction producing charge and field concentration^{33,34}.

Should electrostatic repulsion increase the bubble radius a factor of 3.3 during transit, limiting sonic velocity 300 ms^{-1} would occur in a 30 kV m^{-1} breakdown field at about 35 km. Also, with $1,000 \text{ MV}^{5,35}$ cloud-ionosphere potential spread equally over 10-m gaps between 3,000 bubbles, we find breakdown is exceeded at a similar altitude. As these two views apparently have a similar limitation, we assume that a temporary charge layer could form at 35 km. This might explain a large decay time constant in one set of observations^{7,8}. Above 35 km, a steady-state filament may be more like the indefinitely extendable positive column of a gas discharge³⁶, with sonic velocities or other departure from equilibrium limited by oscillation.

A charge layer at (say) 30-km altitude would have about 0.5 C km^{-2} if it shielded 0.3 kV m^{-1} above from 60 kV m^{-1} below. If the layer is 3 km thick, the excess charge density is about 100 times that typical at 70 km for (presumed) electrostatic equilibrium. This could give 900 MV at 60 kV m^{-1} across the lower 15 km and 12 MV at 0.3 kV m^{-1} across the upper 40 km. Now, if conduction avalanches along the upper part of the filament, the lower 900 MV could jump suddenly (in 20 ms) by more than 10%, possibly causing electron runaway and X rays³⁷ to complete the breakdown with upward lightning^{17–25}.

Because it is a good source of ionizing bremsstrahlung X-ray bursts which have been observed to penetrate to 30 km altitude¹⁴, the magnetosphere is actually a quite plausible trigger source in this situation. Delay between the start of a synchronous 'echo' (Figs 1 and 2) and any triggered whistler is probably due mostly to the magnetospheric processes^{12,14,38}.

Many other details considered in terms of the current statement of the problem also recommend a type of phase-change explanation. The extent of any departure from equilibrium is a primary question. Reference 9 gives insight into the history of the topic. A positive column structure³⁶ may be an essential feature, and its triggering could play a more general role, for example in 'sympathetic' lightning³⁹ or as a supplement to solar

ionization in Sun-weather coupling^{35,40}.

The author thanks his colleagues for their help and the NSF Division of Polar Programs for support.

Note added in proof. There exists a model⁴², previously unknown to the author, which extends Wilson's picture⁵ somewhat; another view⁴³ suggests how phase changes in the electrical medium, possibly of central importance to some observations^{6–9,16}, might occur in the atmosphere. Also, some experiments⁴⁴ have shown how discharges may respond to space-charge 'bubbles'.

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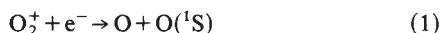
The production of O(¹S) from dissociative recombination of O₂⁺

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The suggestion that the dissociative recombination (DR) of O₂⁺ with an electron could be an important process in the Earth's upper atmosphere first appeared in the literature over 55 years ago¹. In 1947, Bates and Massey² pointed out that DR of O₂⁺ was the only process that could explain the observed electron recombination rates in the E and F1 regions of the ionosphere. In 1954, Nicolet³ proposed that DR of O₂⁺ was the source of the ionospheric emission at 5,577 Å in the Earth's airglow. In the intervening years, the DR of O₂⁺ leading to O in the excited ¹S state has been the subject of several reviews and many papers^{4,5} and has been termed the 'classical'⁶ ionospheric source of the well-known green line emission. Nevertheless, the rate coefficient for DR leading to O(¹S) has never been reliably determined in the laboratory. Here, we report the first theoretical calculations of the rate coefficient for a wide range of temperatures.

The DR of O₂⁺ with an electron leading to excited atomic oxygen in the ¹S state is described by



For the low vibrational levels of the ion ($v < 10$), the products of reaction (1) can be described by a single potential energy curve of ¹Σ_u⁺ symmetry⁷. In agreement with earlier theoretical studies⁷, analyses of the Atmosphere Explorer E satellite measurements have shown a correlation between the quantum yield of O(¹S) from reaction (1) and the extent of vibrational excitation of the ion although the vibrational distribution was unknown⁸. Dissociation on a surface leading to O(¹D) + O(¹S) has been supported by interferometer emission-line profile measurements at 5,577 Å taken by the Dynamics Explorer Satellite⁹. The modelling of this process requires knowledge of the dependence of the DR rate coefficients for production of ground and excited atoms on ion vibrational excitation and electron temperature. Except for the total DR rate coefficient to all channels^{10–13}, reliable rate coefficients and quantum yields leading to specific excited states of O from individual vibrational levels have not been previously available.

The mechanism¹⁴ for DR is illustrated in Fig. 1. The ion in vibrational level v captures an electron of energy ϵ into a neutral repulsive state which can dissociate before the captured electron is emitted. The probability of capture into vibrational level v is proportional to a Franck-Condon (FC) factor between a bound ion vibrational wave function and a continuum wave function which has a small internuclear distance (R) turning point near the tip of the vertical arrow in Fig. 1. As ϵ increases, the turning point of the continuum wave function moves to smaller R , and the FC overlap samples the smaller R region of the bound ion wave function.

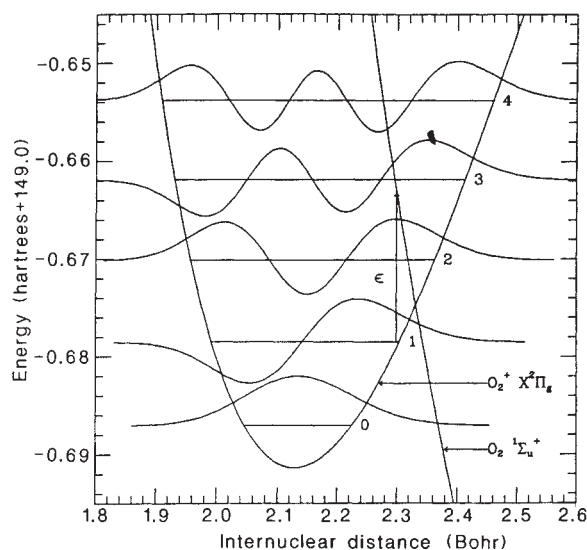


Fig. 1 Potential energy curves for the ground state of O₂⁺ (from ref. 18) and for the dissociative ¹Σ_u⁺ state calculated here. The ion vibrational wave functions for the lowest five levels are each plotted on the same scale.

The orbitals for the large-scale wave functions reported here for the ¹Σ_u⁺ state have been determined in complete active space self-consistent field calculations (CASSCF)¹⁵ over a nuclear-centred contracted gaussian basis set of 6s,3p,2d,1f size. The final configuration interaction (CI) wave functions were expanded in terms of the CASSCF orbitals and generated from a ten-term reference set which contained all the configurations needed to describe the dissociation of ¹Σ_u⁺ to a ¹D and a ¹S oxygen atom. The CI involved all single and double excitations to the full virtual space and yielded a 139,946-term wave function. The SWEDEN group of programs (written by C. W. Bauschlicher, P. E. M. Siegbahn, B. Roos, P. Taylor and J. Almhof) and the direct CI method of Siegbahn were used to determine the large-scale wave functions¹⁶. The final energies, corrected for the missing quadruple excitations¹⁷, were determined at 58 points in the range 1.85–8.0 a₀ (Bohr). The resulting potential curve for ¹Σ_u⁺ is shown in the region near the ion in Fig. 1. The ground-state ion potential curve is the RKR (Rydberg, Klein, Rees) curve¹⁸ positioned at the experimental ionization potential above the calculated ground state and shifted by 0.0180 a₀ to larger R , to compensate for the difference between the calculated and experimental equilibrium R .

The cross-section, $\sigma(v)$, for DR through ion vibrational level v , was calculated using the expression derived by Giusti¹⁹ in combination with numerically determined vibrational wave functions obtained by solving the one-dimensional nuclear Schrödinger equation in the ¹Σ_u⁺ and ion potentials. The cross-section is approximately directly proportional to an electronic width, Γ , which couples the electronic continuum of the free electron and ion to the repulsive dissociating resonance state. The width, a matrix element of the hamiltonian operator, is calculated using Fermi's Golden Rule. High principal quantum number Rydberg orbitals are determined in improved virtual orbital²⁰ calculations using an extended Rydberg basis set and are used to represent the free electron. The calculation of Γ is then a bound-state problem. The multi-configuration wave function for the ion plus high Rydberg orbital (or free electron) is optimized in the field of an optical potential in a procedure based on the use of Feshbach²¹ projection operators (S.L.G., manuscript in preparation). Γ is determined for several values of the principal quantum number, n , of the Rydberg state and a threshold value is determined by extrapolating to $n \rightarrow \infty$. The resulting Γ calculated for the ¹Σ_u⁺ state of O₂ is 0.29 eV. The rate coefficient, α , is then obtained by averaging the cross-sections over a Maxwellian distribution of electron energies.

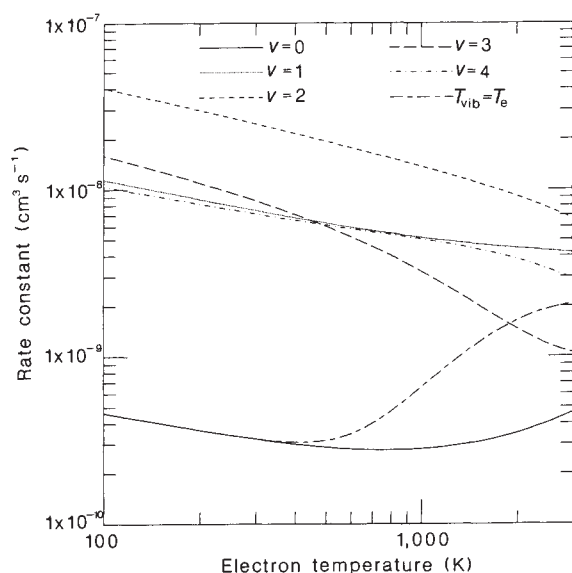


Fig. 2 The calculated rate constant for dissociative recombination along the $1\Sigma_u^+$ dissociative route for each of the lowest five vibrational levels of the ion and for the case where the vibrational and electron temperatures are identical. In each case, the ion rotational temperature is equal to the electron temperature.

The calculated rate coefficients are shown in Fig. 2. The values of α in the electron temperature (T_e) range $200 < T_e < 400$ K can be closely represented by $\alpha(v=0) = 3.2(+1.6, -1.1) \times 10^{-10} \times (T_e/300)^{-0.34}$, $\alpha(v=1) = 7.5(+1.9, -1.7) \times 10^{-9} \times (T_e/300)^{-0.41}$, $\alpha(v=2) = 2.5(+0.3, -0.4) \times 10^{-8} \times (T_e/300)^{-0.45}$, $\alpha(v=3) = 8.6(+5.6, -4.3) \times 10^{-9} \times (T_e/300)^{-0.60}$ and $\alpha(v=4) = 7.2(+1.4, -2.3) \times 10^{-9} \times (T_e/300)^{-0.36}$. The values for α are for equal ion rotational and electron temperatures. The precision estimates are the average uncertainties that result for an estimated $\pm 15\%$ uncertainty in the electronic width and a ± 0.1 eV uncertainty in the position of the dissociative $1\Sigma_u^+$ route relative to the ion. The $v=0$ level has the lowest α and is a factor of 78 lower than the highest α due to $v=2$. Clearly, for the T_e range shown in Fig. 2, the rate coefficient from $v=2$ is an upper bound to the coefficient at any vibrational temperature, T_{vib} . The relative magnitude of these values can be understood by inspection of Fig. 1. At low electron energies, the FC factor between the continuum vibrational wave function of $1\Sigma_u^+$ and ion vibrational level v increases with increasing v for $v \leq 2$. For $v=0$, the FC factor increases with electron energy leading to α rising above 790 K. The increase is due to the peak in the cross-section at 1.9 eV which is a 'reflection' of the shape of the $v=0$ vibrational wave function. Bardsley²² has shown that a variation of the rate coefficient as $T_e^{-0.5}$ arises if the FC factors are constant with electron energy. For $v=0, 1, 2, 4$ in the calculations reported here, the FC factors increase as the electron energy increases from zero and the rate coefficients have an exponent which is more positive than -0.5 .

Using calculated rates for each of the lowest 10 vibrational levels of the ion, values of α for the equilibrium case in which the electron and vibrational temperatures are identical have been determined and are shown in Fig. 2. For $T < 300$ K, the rate coefficient is indistinguishable in Fig. 2 from the coefficient for $v=0$. For $T > 300$ K, the α rises due to the increasing contribution of the $v > 0$ rate coefficients to the total rate coefficient and to the increase in the $v=0$ rate coefficient with increasing T_e . For $800 < T < 1,500$ K, the rate coefficient is closely represented by $\alpha = 8.1 \times 10^{-10} \times (T/1,150)^{1.47}$. It is interesting to note that atmospheric models^{4,5,8,9} have all used a negative exponent for the temperature dependence of the rate coefficient near 1,000 K. The positive exponent obtained here for $T_e = T_{vib}$ near 1,000 K is confirmation that the atmospheric models must be describing conditions for which $T_e \neq T_{vib}$. The

most recent atmospheric model⁸ reports the rate coefficient to be $(0.83-2.0) \times 10^{-8} \text{ cm}^3 \text{ s}^{-1}$ for T_e in the range 680 K–840 K. The results obtained here indicate that the derived atmospheric coefficients must pertain to O_2^+ which cannot be characterized by a Boltzmann temperature but which is instead significantly populated in the $v > 0$ levels.

The above results indicate that a value for the quantum yield of $\text{O}(\text{S})$ is meaningful only if the ion vibrational distribution is specified. Taking the ratio of the DR rate coefficient reported here for $v=0$ to the laboratory value¹⁰ of the total rate coefficient at $T_e = T_{vib} = 300$ K gives a quantum yield of 0.0016 (+0.0009, -0.0005) for $\text{O}(\text{S})$ from $v=0$ and should be compared to the original widely used quantum yield of 0.1²³. The quantum yield reported here supports the more recent view²⁴ that in the earlier experiment²³ the ions may not have been vibrationally relaxed. However, analysis of the experiments^{23,24} is difficult due to the recent measurement²⁵ of the rapid relaxation of vibrationally excited O_2^+ in an argon buffer. The calculation of the quantum yields from each of the higher vibrational levels is currently in progress.

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Note added in proof: In agreement with the conclusions reported here, a new model of the 5,577 Å emission recorded by the Dynamics Explorer satellite shows that the $v=1$ and 2 ion vibrational levels are the dominant contributors to $\text{O}(\text{S})$ production²⁶.

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Plume formation in the D''-layer and the roughness of the core-mantle boundary

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Numerical simulations of the formation of thermal plumes in the D''-layer at the base of the Earth's mantle show that plumes are initiated by coalescence of small-scale convective instabilities within the low viscosity region immediately above the core-mantle

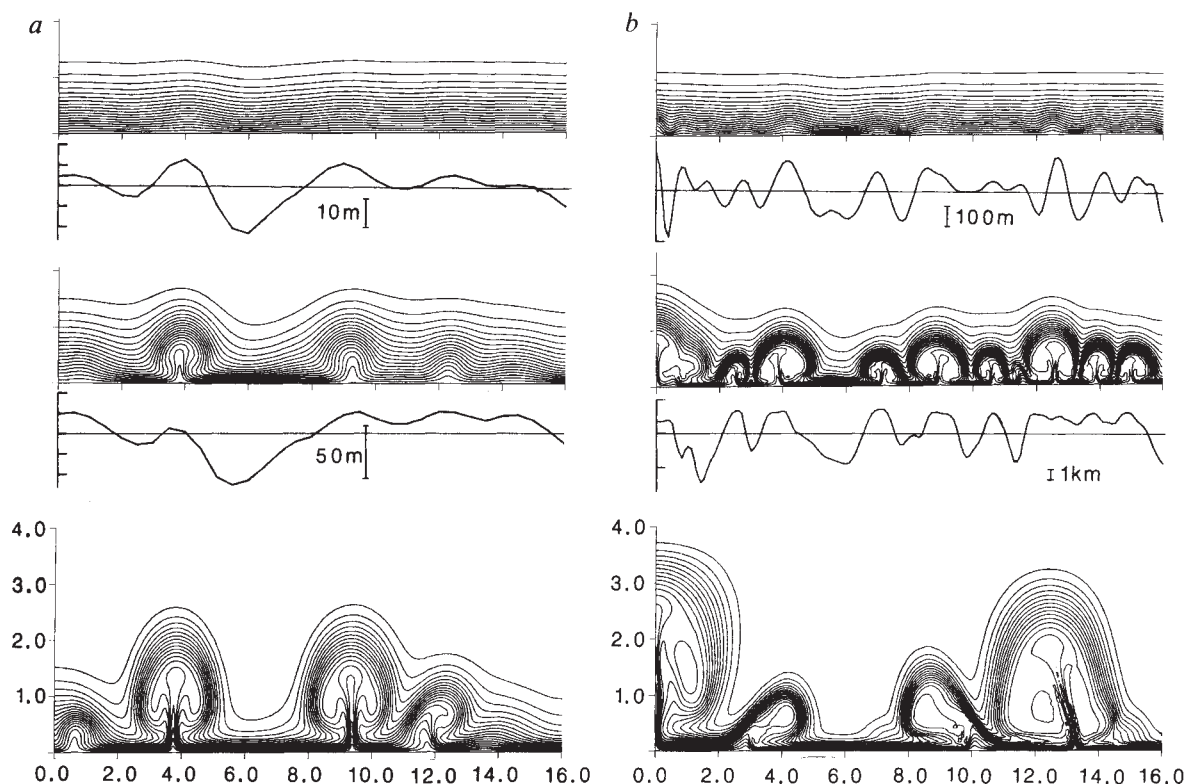


Fig. 1 Temperature contours and core-mantle boundary (CMB) topography in two simulations of the growth of convective instabilities in the D'' thermal boundary layer with thermally activated viscosity. Contour interval is 40 K; vertical and horizontal axis units are 100 and 200 km respectively. The CMB topography scale is given by vertical bars. Case *a*, thermal boundary layer structure and CMB topography with 10^3 viscosity contrast at 64, 83 and 93 Myr. Case *b*, thermal boundary layer structure and CMB topography with 10^4 viscosity contrast at 47.6, 63.5 and 79.4 Myr. Other model parameters are given in Table 1.

boundary (CMB). These instabilities support topographic roughness on the CMB having horizontal scales of 20–50 km and provide a source for scattered P-waves seen as precursors to the phases PKIKP and PKKP. The calculated structure of fully developed plumes emerging from D'' consists of 5–50 cm yr⁻¹ flow confined to 50–100 km thick vertical conduits. With strongly temperature-dependent viscosity, plumes exhibit time-dependent behaviour, including upward propagating solitary conduit waves, which may contribute to episodicity in hotspot volcanism.

Thermal plumes, produced by instabilities from hot boundary layers in the mantle, have long been proposed as the ultimate cause of hotspot volcanism¹. There are only two plausible locations for the hot boundary layer—the transition zone² near 670 km and the D''-layer just above the CMB^{3–5}. The arguments used against D'' as the plume source follow from the interpretation of the 670-km transition as a compositional discontinuity⁶ which would suppress mass transport from the lower mantle. However, recent evidence for aseismic extension of subducted slabs below 670 km (ref. 7) indicates that the transition zone may not be an impermeable barrier to plumes originating in the deep mantle. The D'' layer is particularly attractive as a source region because estimates of CMB temperatures ($4,400 \pm 600$ K)⁸ and lower mantle temperatures ($2,900 \pm 200$ K)⁹ require the presence of a strongly superadiabatic boundary layer.

Seismological data indicate highly anomalous vertical and lateral structure in D'', compared to the rest of the lower mantle. The radially averaged structure includes a thin (<100 km) basal low velocity¹⁰ and high attenuation¹¹ zone that can be explained by an 800 K thermal boundary layer¹², plus a possible discontinuity at 280 km above the CMB defined by increased S-wave velocity¹³ but not well defined by P waves¹⁴ that may represent an accumulation of subducted slab material. Lateral variations in D'' are roughly comparable to vertical variations. On the largest scales, lateral variations of 2% in velocity¹⁵ and ± 6 km in CMB topography³³ have been inferred from body wave

tomography. Regional-scale studies have demonstrated the existence of heterogeneity with 150–500 km lateral dimensions¹⁶.

Particularly significant for the plume hypothesis are the observations of PKIKP and PKKP precursors, which are interpreted as scattering from roughness on the CMB^{17–22}. The identification of PKKP precursors as back-scattered signals implies that the scatterers are boundary roughness elements and not volume heterogeneity in the mantle. Roughness elements approximately 200 m in height with half-widths of 10–40 km are sufficient to account for the observed scattering²³, although the amount of scattering is regionally variable, indicating a similar variability in boundary roughness²⁴.

Since the viscous relaxation time constant for unsupported CMB topography is certainly less than 10^6 yr, a dynamic mechanism is required to explain its presence, its lateral scales and its regional variability. The presence of small-scale roughness indicates the existence of very localized stress fields. Linear stability analyses of a D'' thermal boundary layer with temperature dependent viscosity²⁵ demonstrate that if the viscosity drop through the layer exceeds about 10^3 , convection confined to the low viscosity sublayer can occur. Numerical experiments on finite amplitude instabilities²⁶ confirm this effect and show that groups of small convection cells merge to form plumes, suggesting that the seismically inferred roughness may be supported by boundary layer convection, which in turn leads to formation of plumes.

We have explored these ideas further with a series of very high resolution finite element calculations of plume formation in D'', for several plausible rheologies and boundary conditions, in order to focus on both the early and later stages of boundary layer development. We examine growth of the D'' boundary layer from an initially homogeneous isothermal state. This is the simplest model which acknowledges the importance of time-dependent effects in shaping the character of mantle plumes and it is a substitute for a more realistic model in which variabil-

Table 1 Material properties for D"-layer simulations

Property	Value	
Lower mantle temperature	2,973 K	
CMB temperature	3,773 K	
Lower mantle density	$5.5 \times 10^3 \text{ kg m}^{-3}$	
Outer core density	$10 \times 10^3 \text{ kg m}^{-3}$	
Thermal expansivity	$3 \times 10^{-5} \text{ K}^{-1}$	
Thermal conductivity	$6.6 \text{ W m}^{-1} \text{ K}^{-1}$	
Specific heat	$1.2 \text{ kJ kg}^{-1} \text{ K}^{-1}$	
Gravity	10 m s^{-2}	
Lower mantle viscosity	10^{22} Pa s	
Model domain	$400 \times 1,600 \text{ km}$	
	Case <i>a</i>	Case <i>b</i>
CMB viscosity	10^{19}	10^{18} Pa s
Activation enthalpy	805	1975 kJ mol ⁻¹

ity in D" structure results from the overall variability in mantle convection. Evidence for intrinsic time dependence in mantle convection includes the observed changes in plate boundary configurations²⁷, and the apparent 200 Myr upper limit on hot-spot duration²⁸. On theoretical grounds, mantle convection is expected to be highly time-dependent with recurring boundary-layer instabilities playing an important role, because of the presence of radioactive heat sources²⁹. The transient heating model is an idealization of a single episode within a recurring pattern of boundary-layer instabilities.

The calculations were made with a two-dimensional primitive variable finite element code for the time-dependent Navier-Stokes equations in the Boussinesq, infinite Prandtl number limit²⁹, in a rectangular domain 1,600 km long and 400 km high with a grid resolution of 5 and 8 km in the vertical and horizontal, respectively. The CMB was represented by a free-slip, impermeable and isothermal lower boundary. Two upper boundary conditions were used: (1) an impermeable top, to examine the onset of boundary-layer convection, and (2) a permeable top, for examining the structure of fully developed plumes. All calculations started from an isothermal state at 2,973 K. At $t = 0$ the CMB temperature was raised by 800 K and maintained. The temperature dependence of viscosity μ was governed by an Arrhenius law of the form

$$\mu = \mu_0 \exp(H/RT) \quad (1)$$

where T is absolute temperature, H is activation enthalpy, R is the gas constant and μ_0 is the rheological coefficient. Calculations were made for two different activation enthalpies, corresponding to viscosity variations of 10^3 and 10^4 across the 800 K temperature jump. The activation enthalpies, plus all other material properties used are given in Table 1.

Figure 1a and b are sequences of temperature contours illustrating the development of convection in D" with viscosity variations of 10^3 and 10^4 , respectively. The resultant CMB topography, shown below, is calculated assuming hydrostatic conditions in the outer core. Similar sequences of events occur for both rheologies; each stage occurs earlier and the scale of the flow is smaller with the larger viscosity contrast. The initial stage is characterized by conductive boundary-layer growth. At 64 Myr (a) and 47.6 Myr (b) instabilities appear in the form of quasi-periodic perturbations confined predominantly to the lowest 50 km (calculations were initialized with a 10 K perturbation applied randomly throughout the flow field). By 83 Myr (a) and 63.5 Myr (b) the perturbations develop into an array of hot, low viscosity diapirs trapped within D" by overlying high viscosity lower mantle material. Note the smaller scale and greater density of diapirs in case b and the corresponding rougher CMB topography. Coalescence of diapirs occurs between this stage and 95 Myr (a) or 79.4 Myr (b), particularly for the higher viscosity contrast. The largest of the trapped diapirs entrain adjacent smaller ones, reducing the number from 10 at 63.5 Myr to 4 at 79.4 Myr. Diapirs that finally detach from

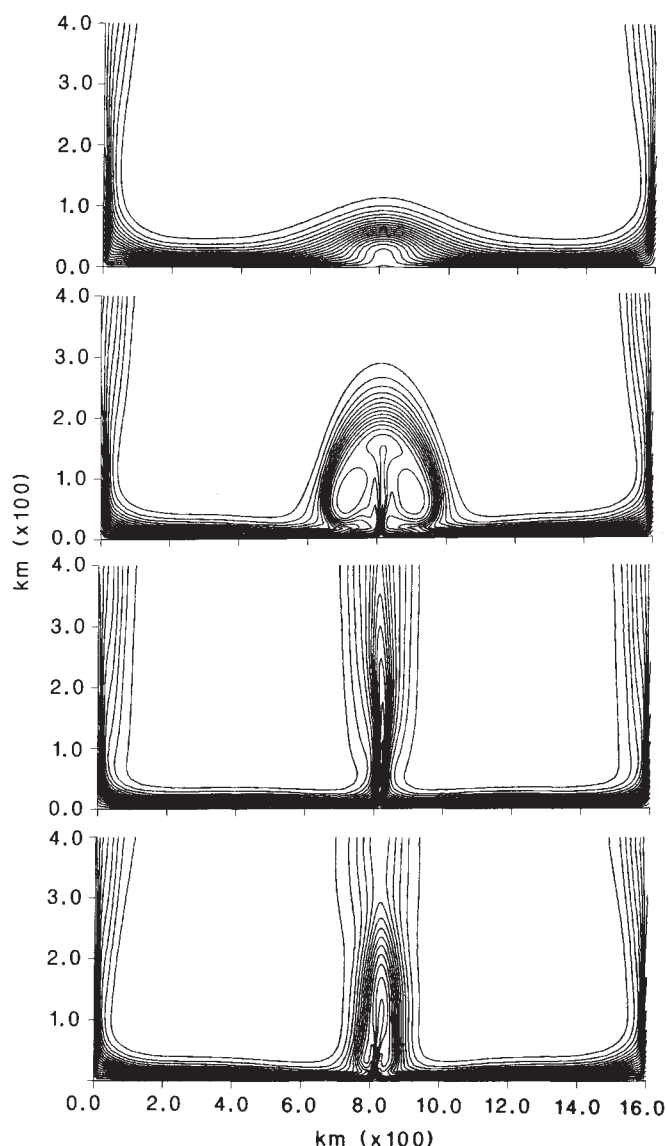


Fig. 2 Temperature contours of time-variable plume and thermal boundary layer structure in the D"-layer at 111, 127, 143 and 175 Myr, computed with a permeable top boundary condition. Temperature contour interval is 40 K; vertical and horizontal scales are 100 and 200 km respectively. Model parameters are those of case b, Table 1.

the boundary layers have nearly the same size in the two cases, indicating a minimum escape size, governed by the rheology of the exterior lower mantle. The rheology of D" controls the scale of boundary-layer convection and dictates the number of small diapirs that must merge in order to achieve the escape size.

The amplitude of CMB topography grows in step with the boundary-layer instabilities and closely reflects, at each stage, the horizontal dimensions of the diapirs. Both the topographic amplitude and its dominant wavenumber increase with increasing viscosity contrast. At 83 Myr in case a, topographic undulations have approximately 50-m amplitude and half-widths (quarter wavelengths) of nearly 100 km. At the corresponding stage in case b (63.5 Myr) the amplitude is nearly 2 km with half-widths in the range 20–50 km. Small-scale convection produces much more topography than previously estimated for the D"-layer⁵.

Deep-mantle viscosity and its temperature dependence are poorly constrained quantities, so it is not possible to decide, on the basis of rheological considerations alone, which of these two cases is more appropriate. But clearly, the topography

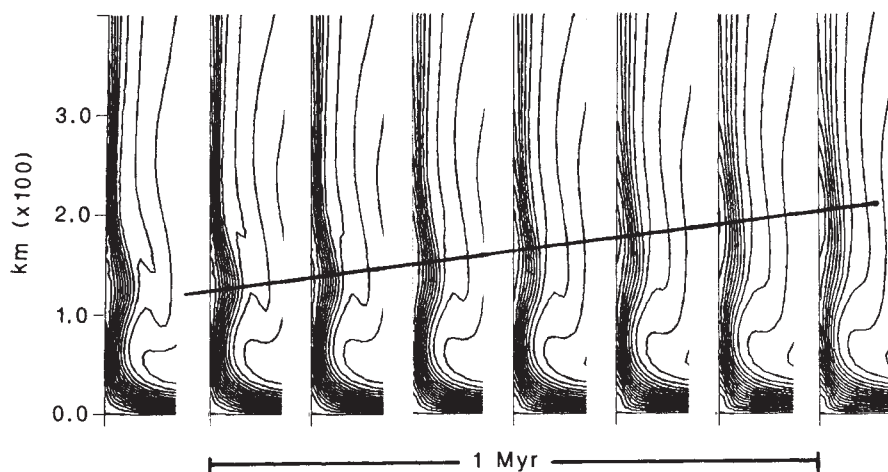


Fig. 3 A detailed time sequence of temperature contours showing the propagation of a dispersive conduit wave, generated near $t = 50$ Myr in a simulation using the same parameters as in Fig. 2. Only the right half of the plume-wave structure is shown. The disturbance core propagates at approximately 7.4 cm yr^{-1} .

produced in case *b* is more representative of CMB roughness as inferred from the scattering observations. Thus, a viscosity contrast of approximately 10^4 or greater across the D'' thermal boundary layer is a requirement for supporting a seismically rough CMB by the mechanism of convective instability. Another characteristic of plume formation with large viscosity contrasts revealed by these calculations is the tendency for diapir mergers, which has the effect of increasing the horizontal scale of boundary topography. The regional differences in P-wave scattering may be a reflection of various stages of boundary layer and plume growth occurring simultaneously but at different locations on the CMB.

During the early stages of plume development, while the instabilities are confined to the low viscosity sublayer, the impermeable top boundary condition exerts little control on the flow, but it becomes increasingly unsatisfactory as the diapirs grow and detach. To examine fully developed plumes, a series of calculations were made with a permeable top boundary. Figure 2 is a sequence of temperature contours for the case shown in Fig. 1*b*, modified by the permeable top. The sequence begins 76 Myr after the two largest diapirs ascended along the side boundaries. The residual thermal structure consists of 50–100 km wide plume conduits connected to a 50-km-thick boundary layer. Instabilities between the existing conduits are inhibited by a descending stagnation point flow. This regime is very similar to a steady-state model proposed by Loper⁵. However, this configuration is not steady or even stable. At 111 Myr an instability has developed midway between the two conduits, by 127 Myr it has grown into a large diapir, and by 143 Myr it has ascended, leaving a trailing conduit. Even this new configuration is not steady. Disturbances generated within the boundary layer repeatedly travel up the conduits, producing fluctuations in discharge within each plume. The motion of a typical conduit disturbance is shown in Fig. 3. There is a clear indication of dispersive propagation: the disturbance core propagates at 7.4 cm yr^{-1} and long wavelength components propagate faster.

The properties of solitary conduit waves with cylindrical symmetry have been established both theoretically and experimentally³⁰, using two-fluid models. Adaptation of the theory to cartesian geometry indicates that the conduit waves shown in Fig. 3 are dispersive, with limiting long- and shortwave propagation speeds

$$c_{\infty} = \frac{g'D^2}{4\nu} \quad (2a)$$

and

$$c = \frac{3g'}{\nu_m Dk^3} \quad (2b)$$

where D is the undisturbed conduit width, g' and ν are conduit fluid buoyancy and kinematic viscosity, ν_m is the matrix viscosity

and k is the disturbance wavenumber. Using 50 km and 500 K for the undisturbed conduit width and temperature excess, and 250 km for the disturbance half-wavelength, equation (2*b*) predicts a propagation speed of 7.85 cm yr^{-1} , in good agreement with Fig. 3. While the disturbance core propagates at a speed dependent on the exterior viscosity, the leading edge propagates at the longwave speed c_{∞} which depends on the conduit viscosity and could be as large as 50–100 cm yr^{-1} . The travel time for long conduit waves across the whole mantle would then be only 4–8 Myr. This is comparable to the time constant for volcanic periodicity along the Hawaiian-Emperor chain³¹. Conduit waves offer a dynamically consistent mechanism for rapid transmission of thermal disturbances from D'' to the lithosphere.

If the D''-layer at the base of the mantle is a thermal boundary layer with a viscosity decrease of about 10^4 or greater, then instability in the layer leads to vigorous small-scale sublayer convection on a timescale of tens of Myr and with horizontal scales of tens of kilometres. Coalescence of the sublayer diapirs, also on a timescale of tens of Myr, leads to plume formation. This interpretation is consistent with lower mantle and core temperature estimates and with the seismic velocity radial structure and attenuation as inferred from body wave seismology; it also provides a source for P-wave scattering by topographic roughness on the CMB. Boundary-layer convection can support topographic elements with half-widths as small as 20 km and amplitudes as large as 2 km. It is altogether possible that D'' contains significant compositional heterogeneity, perhaps in the form of inverse continents³². In addition to providing long wavelength topography, compositional variations would inhibit plume escape from the boundary layer. This would further enhance the importance of small-scale convection and would increase the level of time dependence, fine-scale heterogeneity and CMB roughness over what we have shown.

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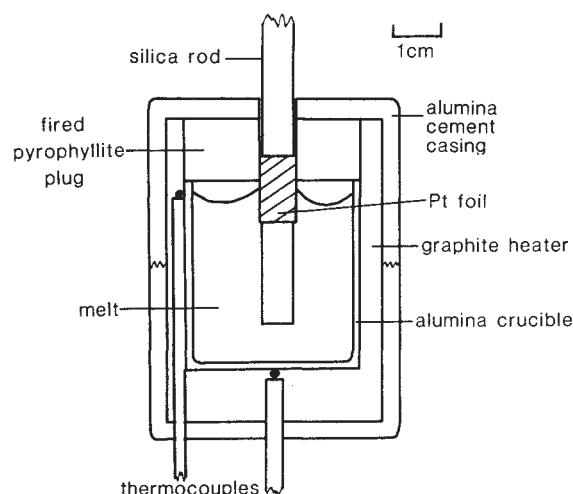


Fig. 1 Schematic cross-section of apparatus used. A layer of platinum foil covering the top of the crucible is omitted.

Compositional convection and layering in a rock melt

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When a crystal grows (or dissolves) in a solution it becomes surrounded by a thin boundary zone of liquid that is relatively depleted (or enriched) in crystal components. The compositional difference and, hence, the horizontal density gradient that exist in this zone, may cause it to convect away from the crystal¹. This process of natural convection is a possible cause of fractionation and liquid layering in crystallizing magmas²⁻⁹. Experiments with aqueous solutions have demonstrated the efficacy of the process in model magma chambers^{4,10}, and recent growth and dissolution experiments involving minerals in rock melts have suggested that compositional convection indeed occurs in silicate systems¹¹⁻¹⁵, but the process has not been demonstrated unequivocally for rock melts. Here we report on simple experiments involving silica dissolution in a superheated mafic rock melt in which compositional layering evidently occurred. The results are consistent with the operation of compositional convection during dissolution, and suggest that more rigorous experiments will be useful in testing some of the new principles of fluid motion recently introduced to petrology.

This work was conceived as an exploratory, pilot programme, using only equipment to hand. The apparatus and technique used were not ideal, resulting in a high proportion of uncompleted experiments (60%), and in limited control over the experimental conditions, which may account for some of the unexpected observations to be mentioned.

A stationary rod of silica glass, 6 mm in diameter, was suspended in an alumina crucible containing molten picrite (Fig. 1, Table 1) at 1,450 °C (~40 °C above the liquidus temperature), for periods from 0.25 to 3 hours. Control of temperature was by manual adjustment of the furnace power. Due to the large thermal mass of the apparatus, control was poor, the temperature varying by ± 25 °C during each experiment. The rod was protected from flux-line attack¹⁶ by a short collar of thin platinum foil at the melt-air interface. Samples were heated in an induction furnace using a carbon sleeve as susceptor. Two thermocouples were used, one touching the base of the alumina crucible, the other touching the side close to its top. The vertical position of the susceptor within the coil that emits radio waves was adjusted until the temperature difference between the thermocouples was less than 15 °C. Runs were quenched in air at ~ 3 °C s⁻¹. Each charge was sliced vertically through the undissolved rod, and a slice obtained for electron probe analysis of the quenched melt. Chips of glass were also removed for measurement of FeO and Fe₂O₃ contents by wet chemical methods. Changes in rod thickness indicate a dissolution rate ($d(\text{radius})/dt$) of 200 ± 20 $\mu\text{m hr}^{-1}$.

While the quenching rate was fast enough to form glass, it was nonetheless rather slow, taking approximately 2.5 min to reach the glass-transition temperature. During this time it is possible that thermal convection of the melt may have occurred, causing mixing (see later). The obvious alternative of plunging the charge into water was tried but abandoned because the

Table 1 Compositions of picrite and glasses (run 7)

	Picrite*	Brown glass†	Green glass‡	Glass 5 μm § from rod	Glass 5 μm from crucible
SiO ₂	44.0	44.2	47.4	57.1	40.5
TiO ₂	0.3	0.3	0.2	0.1	0.2
Al ₂ O ₃	15.1	15.2	14.7	11.7	21.6
Fe ₂ O ₃	1.5	0.2	—	—	—
FeO	8.1	9.3	9.2	7.2	9.8
MgO	19.2	19.0	17.4	14.0	16.7
CaO	9.0	8.9	7.8	6.8	8.0
Na ₂ O	1.2	1.1	1.2	1.9	1.3
K ₂ O	0.1	0.1	0.1	0.1	0.1
	98.5	98.3	98.0	98.9	98.2

* X-ray fluorescence analysis and titrimetric measurement of Fe²⁺. Recast H₂O free.

†‡ Electron probe analysis and titrimetric measurement of Fe²⁺; † middle of brown glass in traverse A (Fig. 2); ‡ in middle of green glass in traverse A.

§|| Electron probe analysis at either end of traverse E. All iron reported as FeO.

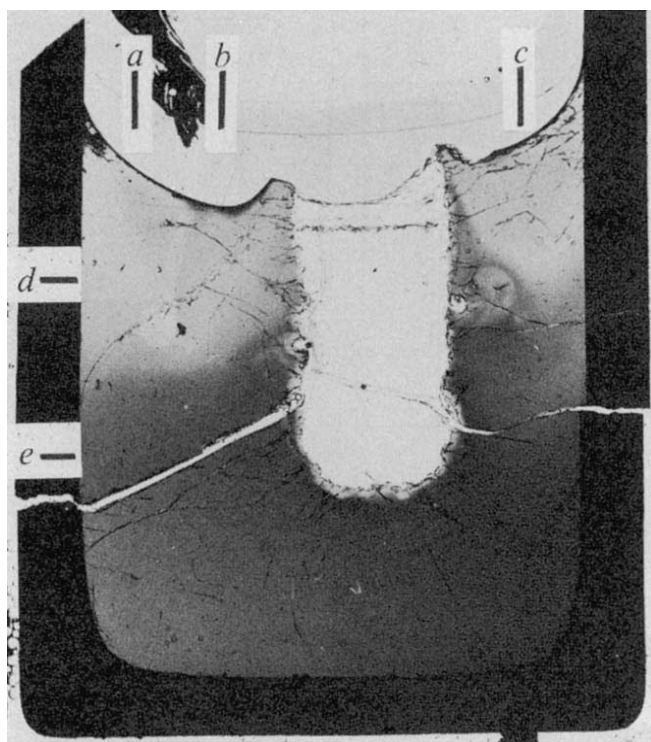


Fig. 2 Thin section of the product of experiment 7 (40 min duration). Crucible internal diameter is 1.5 cm. Brown glass (darker grey) occupied the lower two-thirds of the charge and green glass (lighter grey) the upper portion. Note how the brown-green glass junction bends upwards near the silica rod, perhaps caused by viscous drag. *a-e* indicate the locations of electron microprobe analyses in line traverses of the section (Fig. 3).

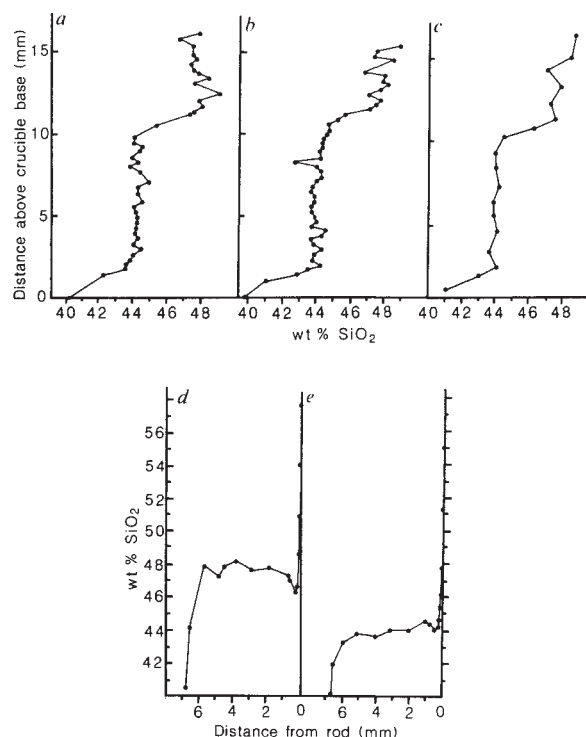


Fig. 3 Variations in SiO_2 content of the glass in the five line traverses shown in Fig. 2.

whole assemblage violently fragmented, making the run product unusable.

Another deficiency of the technique used was the inability to control oxygen partial pressure. Combustion of graphite evidently allowed carbon monoxide to reduce ferric iron in the melt which has the benefit that the colour of the glass changes from brown to bottle green. In the five completed experiments the quenched melt was stratified with respect to colour and composition: bottle-green, silica-enriched glass overlies brown glass, the latter differing only slightly in composition from the picrite (Fig. 2, Table 1). The longer an experiment, the thicker the green layer.

Chemical analyses of chips of glass show that whereas the brown glass contains both Fe^{2+} and Fe^{3+} , the green glass has only Fe^{2+} . (Indeed the data indicate that it may even contain a small quantity of metallic iron but no metal particles are seen.) Clearly, the iron in the melt at the top of the charge was reduced during an experiment. Whereas the run product of an experiment conducted for 0.65 h has a layer of reduced glass up to 6 mm thick, that of a second experiment run without a rod but for the same duration has a reduced layer just 0.1 mm thick. It follows that the presence of the rod must have caused flow of the underlying, relatively oxidized melt to the top of the charge where it was reduced.

In a polished thin section of the product of experiment 7 (Fig. 2), numerous glass analyses in line traverses, obtained by an electron microprobe, show the existence of horizontal composition gradients of $\sim 300 \mu\text{m}$ thickness adjacent to the silica rod and to the crucible wall; these are enriched in Si and Al, respectively (Fig. 3, Table 1). Vertical compositional boundary layers clearly formed due to dissolution. The traverses also show the distinction between the green and brown glasses with respect to SiO_2 content, when measured at a distance from these vertical

boundary layers. Whereas the brown glass is apparently homogeneous, the green glass tends to be more variable in composition (Fig. 3). The glasses are separated by an ill-defined 900–1,300- μm thick region through which colour and composition change gradually.

The compositional differences between the green and brown glass layers and the relative volumes of the two glasses in run 7 (Fig. 2, Table 1) indicate that, if the green glass has acquired SiO_2 from dissolution of only that part of the rod now immersed in green glass, then it should contain just 45.1 wt % SiO_2 . The actual figure of ~ 47.4 wt % means that some of the silica dissolved from that part of the rod still surrounded by brown glass has moved upwards into the green layer. It is estimated that 70% of all the dissolved SiO_2 from the rod was incorporated in the upper (green) layer.

The fluid flow necessary to transfer dissolved silica upwards presumably arose from the density difference between picritic melt and the vertical layer of silica-enriched melt that surrounded the dissolving rod. Computed densities^{17,18} for the green and brown melts and for the melt within $5 \mu\text{m}$ of the rod are 2.69, 2.73 and 2.59 g cm^{-3} , respectively, consistent with buoyant ascent of the silica-enriched melt surrounding the rod. The formation of the steadily thickening silica-enriched layer at the top of the crucible would seem to be an example of the 'filling-box' mechanism of producing liquid stratification^{10,19,20}. The theory of the mechanism predicts that the upper layer should display a downwards composition gradient, with the most siliceous melt at the top of the layer. Whereas analyses of the green glass in traverses B and C display trends indicating existence of a slight compositional gradient (Fig. 3), and hence consistent with the filling-box mechanism, those in traverse A show no such trend. It is conceivable that thermal convection during the protracted quenching of the experiment has reduced and even eliminated original compositional gradients in the green glass.

Upward laminar flow of the vertical boundary layer of melt adjacent to the rod ought to have caused downward tapering of the rod, as observed in other systems^{1,21,22} and thinning of

the boundary layer²³. In the present experiments tapering is not pronounced and is confined to the lowermost part of the rod (Fig. 2). Tapering here may only have resulted because the initially sharp lower edge of the rod extended further into the adjoining composition gradient in the liquid than other parts of the rod (Table 1), and so it was bathed in relatively more undersaturated melt²⁴. The electron probe was used to measure the thickness of the vertical boundary layer by line traverses of the glass at right angles to the rod, while monitoring the Si X-ray count rate on a strip chart. Values of $300 \pm 30 \mu\text{m}$ were obtained but these showed no systematic thinning of the layer from top to bottom of the rod. While this feature might again be ascribed to thermal convection during quenching having erased any variation in thickness, when coupled with the lack of clearcut evidence of rod tapering, it suggests that no pronounced variation in boundary layer thickness developed during experiments.

These rudimentary experiments demonstrate compositional convection and layering in picritic rock melt and hence support the view that compositionally zoned magma chambers can develop when such a magma dissolves siliceous country rocks²⁵⁻²⁷. The same might be expected to happen during magma solidification on the walls of a chamber, when growing crystals remove components from liquid, reducing its density compared to the far-field magma^{3-8,20}. This demonstration shows that there is a role for experimental petrology in the investigation of magmatic fluid processes. We plan to use better-controlled experiments to examine the influence of time on compositional evolution of the glasses, to establish the pattern of the fluid

motions, to examine whether this mechanism of solute transport also operates in more viscous melts, to investigate other geometric arrangements of the dissolving solid, and to seek evidence of convection during crystal growth.

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A DNA sequence specific for forest form *Onchocerca volvulus*

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Onchocerciasis, or river blindness, is caused by infection with *Onchocerca volvulus*, a filarial parasite which infects about 40 million people in Africa and Latin America. Epidemiological¹, clinical², entomological^{3,4} and serological⁵ studies of African onchocerciasis led to the hypothesis that *Onchocerca volvulus* exists in different forms in the forest and savannah. It is uncertain if these differences are due to genetic differences within *O. volvulus* itself, or to epigenetic factors, such as differences in the host populations. To date no basic biochemical differences between the forest and savannah populations of *O. volvulus* has been found, although isoenzyme studies have shown that differences in allele frequency between forest and savannah populations exist^{6,7}. Here we describe the isolation of a DNA sequence that seems to be specific for the forest form of *O. volvulus*, the first indication of a basic genetic difference between the savannah and forest forms.

To identify DNA sequences which are specific for the forest form of *O. volvulus*, a library consisting of *Sau* 3A restriction

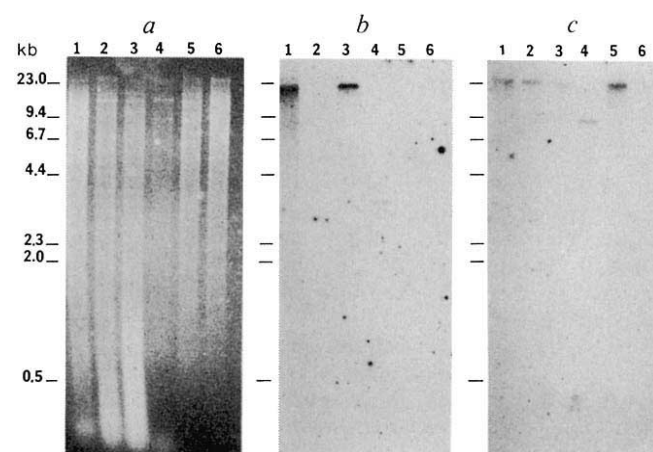


Fig. 1 The clone pFS-1 hybridizes specifically to forest-form *O. volvulus* DNA. *a*, Ethidium bromide staining pattern of one half of the agarose gel. *b*, Southern blot probed with pFS-1. *c*, Southern blot probed with pCL-1. In each panel: lane 1, forest-form *O. volvulus* DNA; lane 2, savannah-form *O. volvulus* DNA; lane 3, *O. volvulus* DNA from the transition region of Togo; lane 4, *Ascaris suum* DNA; lane 5, *Dirofilaria immitis* DNA; lane 6, human DNA. **Methods.** Samples (2 μg) of genomic DNA were digested with the restriction enzyme *Hind*III. A small aliquot of each sample was removed and combined with 100 ng bacteriophage λ DNA to demonstrate that the digestions went to completion. Following the digestion, each sample was divided in half, and each half loaded on duplicate sets of lanes on an agarose gel. After electrophoresis, the DNA was transferred to nitrocellulose using the method of Southern¹⁰. The filter was then cut in half, to produce two duplicate Southern blots. One blot was hybridized with radioactively labelled pFS-1 DNA. The other blot was hybridized with radioactively labelled plasmid pCL-1¹¹ which contains a malaria rRNA gene which hybridizes well to helminthic rRNA genes⁹. This demonstrates that the lack of hybridization of the DNA samples in panel *a* is not due to poor transfer. Following hybridization, the blot hybridized to pFS-1 was washed three times in $0.05\times\text{SSC}$, 0.5% SDS at 54 °C, whereas the blot hybridized to pCL-1 was washed three times in $0.3\times\text{SSC}$, 0.5% SDS at 50 °C.

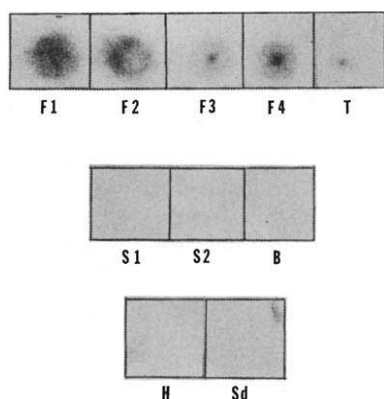


Fig. 2 The cloned DNA of pFS-1 is represented in several isolates of forest-form *O. volvulus* genomic DNA. DNA samples as follows: F1, F2, F3, independent forest-form *O. volvulus* DNA preparations from Grand Bassa County, Liberia; F4, forest-form *O. volvulus* DNA from Bong County, Liberia; T, *O. volvulus* DNA from the transition region of Togo; S1, S2, independent *O. volvulus* DNA preparations from the savannah region of Mali; B, *O. volvulus* genomic DNA from the Yanomami focus in Brazil; H, human DNA; Sd, *Simulium yahense* DNA. Subsequent re-hybridization of this filter to the malaria ribosomal DNA clone demonstrated that DNA was bound to the sites on the filter where the savannah-form *O. volvulus* samples were applied.

Methods. Samples of genomic DNA were added to 0.5 M NaOH, 1.5 M NaCl and incubated for five minutes at room temperature. The samples were neutralized by the addition of an equal volume of 3 M Tris-HCl, pH 8.0, and applied to a nylon membrane using a vacuum filtration apparatus. The filter was baked for two hours at 70 °C, and hybridized to nick-translated pFS-1 DNA. Following hybridization, the filter was washed three times in 0.05×SSC, 0.5% SDS at 54 °C.

fragments of genomic DNA from *O. volvulus* from the forest of Liberia was constructed in the plasmid vector pUC13. Approximately 15,000 colonies were screened for hybridization to genomic *O. volvulus* forest DNA, and lack of hybridization to *O. volvulus* savannah DNA or to human DNA. The clone which appeared most specific for *O. volvulus* forest form DNA was designated pFS-1.

The plasmid pFS-1 hybridizes strongly to a large fragment generated by digestion of *O. volvulus* forest form genomic DNA with the restriction enzyme *Hind*III (Fig. 1b, lane 1) and to a similar band in *O. volvulus* genomic DNA from a transition zone between forest and savannah (Fig. 1b, lane 3). But pFS-1 does not hybridize to *O. volvulus* DNA from the savannah region of Mali (Fig. 1b, lane 2), *Dirofilaria immitis* DNA (Fig. 1b, lane 5), human DNA (Fig. 1b, lane 6), or *Schistosoma mansoni* genomic DNA (data not shown). A single band of hybridization is detected in *Ascaris suum* genomic DNA (Fig. 1b, lane 4), but control experiments have shown that this band is detected if *A. suum* genomic DNA is probed with the vector plasmid alone.

As shown in Fig. 2, pFS-1 hybridizes strongly to four independent isolates of *O. volvulus* DNA from two geographically distinct regions from the forest of Liberia. All the forest DNA preparations shown in Fig. 2 are different from the forest DNA used to prepare the library. Thus, the DNA represented in pFS-1 is found in five independent forest-form DNA isolates, and one DNA isolate from the transition region of Togo. The DNA sequence in pFS-1 therefore is not restricted to a single worm, or worms from a small geographic region. In contrast, pFS-1 does not hybridize to DNA from either of two independent isolates of savannah-form *O. volvulus* (Fig. 2). Further evidence that pFS-1 is highly specific for the forest form of *O. volvulus* is the lack of hybridization to *O. volvulus* DNA from an isolated focus in northwestern Brazil (Fig. 2). In addition, pFS-1 did not hybridize to DNA of the blackfly *Simulium yahense*, a vector for *O. volvulus* in the forest of Liberia (Fig. 2). It should therefore

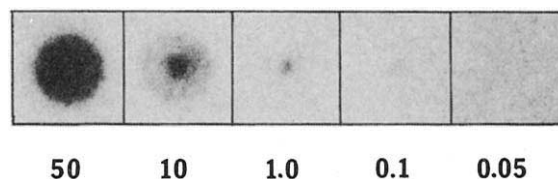


Fig. 3 Sensitivity of pFS-1 for *O. volvulus* forest-form genomic DNA. Various amounts of forest form genomic DNA (shown in ng) were treated and applied to a nylon membrane, as in Fig. 2 legend. The filter was hybridized and washed as described in Figure 2. No hybridization was detected to 400 ng of savannah-form *O. volvulus* DNA applied in a separate well of the same filter, demonstrating the specificity of the hybridization of pFS-1 to *O. volvulus* forest form DNA.

be possible to use pFS-1 to identify forest-form *O. volvulus* DNA in extracts containing either blackfly or human DNA.

There is a great need for a technique for distinguishing between the forest and savannah forms of *O. volvulus*. The Onchocerciasis Control Program (OCP), an international effort directed at reducing transmission of *O. volvulus* primarily through control of the vector, has concentrated its efforts in the savannah where the disease, by its severity, represents a public health problem. The ability to tell if new outbreaks of infection within the control region are due to invasion from the forest or recurrence of the more pathogenic savannah form is therefore essential to the OCP. To use pFS-1 in this manner, it must be capable of detecting small amounts of *O. volvulus* forest-form DNA. As shown in Fig. 3, pFS-1 can detect 1 ng of *O. volvulus* forest-form genomic DNA. Preparation of DNA from adult worms yields 1–10 µg of genomic DNA per adult female. Thus, pFS-1 is clearly sensitive enough to categorize a single adult worm. Furthermore, studies with a previously identified *O. volvulus*-specific probe of comparable sensitivity⁸ have shown that this level of sensitivity is sufficient to detect DNA contained in one infective larva.

To determine if the inserted DNA in pFS-1 has similarities to any previously known sequences, the DNA sequence was determined (Fig. 4). It consists of 153 nucleotides, and contains no direct or inverted repeats. No significant similarities to pFS-1 were identified in the GenBank sequence library.

The isolation of a DNA sequence specific for the forest form in pFS-1 is the first direct demonstration of a basic genetic difference between forest and savannah *O. volvulus*. It is not yet possible to relate the difference represented by pFS-1 directly to the clinical and vectorial differences noted in onchocerciasis in the forest and savannah. Further testing of pFS-1 on many other independent isolates is required, both to demonstrate the generality of the probe, and to establish any correlation to the more significant differences. However, the demonstration that a genetic difference does exist means that the more important traits that distinguish forest and savannah forms of onchocerciasis may not be solely due to epigenetic factors. In addition, the clone pFS-1, when used in conjunction with previously identified DNA probes specific for *O. volvulus*⁹, may form the basis of an assay which will specifically identify the parasite, and then distinguish the forest and savannah forms. Such an assay should have enormous value in monitoring new *O. volvulus* outbreaks within the control region of the OCP, as well as serving as a valuable tool in epidemiological studies of onchocerciasis.

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-GATCATAGGT10 CATCAGTTCT20 TAATTAATT30 TGCAAAC40 GCTGTCGC50
GGAAAAATCG60 CGGCATAAAT70 CTGCAAAAT80 ACCAAAAAT90 AGTCGAATAT100
TTTTTTTAGC110 ACTCAATTG120 AAGGTATGTA130 CCGCTTCTT140
GAAATAGGG150 ATC-

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Fig. 4 Sequence of the inserted DNA of pFS-1. The inserted DNA of pFS-1 was directionally cloned into the single strand bacteriophage vectors M13-mp18 and mp19, and sequenced using the dideoxynucleotide termination method¹². The entire insert was sequenced in both directions.

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Stress ethylene formation determines plant sensitivity to ozone

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Ozone has long been known to cause reductions in the yield of crops¹ although the precise mechanism of how this occurs is still unclear. Recently, ozone has also been suggested as being involved in the forest decline that has occurred in central Europe²—perhaps in combination with other atmospheric pollutants or with various environmental stresses such as chilling, mineral leaching, insect predation or disease. Consequently, it is desirable that the cellular mechanisms by which ozone is toxic to both crops and conifers, and how they might be modulated by other atmospheric pollutants or environmental stresses, are fully understood. Ethylene is normally produced by all plants and in trace amounts it may interact with other plant growth substances to coordinate a wide variety of developmental processes. However, when plants experience environmental stresses they respond by liberating larger amounts of ethylene—often called stress ethylene³. We have been able to demonstrate, using pea seedlings in an experimental fumigation system⁴, that the formation of stress ethylene determines the sensitivity of plants to atmospheric levels of ozone and to suggest how other air pollutants may enhance ozone-mediated leaf injury.

Germinated pea seedlings (*Pisum sativum* L. var. Feltham First) were fumigated with ozone (50–150 p.p.b.v.—parts per 10⁹ by volume—or 100–300 µg m⁻³) for 7 h every day for their first three weeks of growth. Remarkably, after three weeks of fumigation, these plants did not display any visible leaf injury, other than a slight curling of the leaves. By contrast, severe leaf necrosis was developed when three-week-old seedlings grown in clean air were fumigated with similar concentrations of ozone for only one 7-h fumigation period (Table 1, column 4). These two sets of pea seedlings also differed in the amount of ethylene they produced. Rates of ethylene evolution from 1-day-fumigated ozone-damaged plants were more than doubled ($P < 0.01$; column 3), which is typical of the immediate response to ozone that has been reported before for a variety of plants^{5,6}, but the 3-week ozone-fumigated pea seedlings which remained undamaged evolved 92% less ethylene than clean-air-grown seedlings of similar age ($P < 0.01$; column 2).

These two quite different responses to ozone suggest that the rate of ethylene production may have an influence on and modify

Table 1 Ethylene production and leaf injury in pea seedlings

Treatment	Three-week fumigation† (C ₂ H ₄ evolved nmol per g dry weight per h)	One-day fumigation (7 h) (C ₂ H ₄ evolved nmol per g dry weight per h)	Visible leaf injury (%)
Control	2.5 (±0.1)*	2.6 (±0.2)	none
50 p.p.b.v. O ₃	1.9 (±0.2)*	4.7 (±0.2)	0–10
100 p.p.b.v. O ₃	0.3 (±0.1)*	7.0 (±0.4)	10–35
150 p.p.b.v. O ₃	0.2 (±0.1)*	5.7 (±0.5)‡	>50

Ethylene production and degree of leaf injury were assessed in three-week-old pea seedlings either after a 3-week fumigation (7 h every day) with different levels of ozone or after only one 7 h fumigation of 3-week-old, clean air-grown pea seedlings with similar concentrations of ozone. Results represent means (±s.e.m. in parentheses) of at least 7 replicates. Treatments were compared using Student's *t*-test and the 100 and 150 p.p.b.v. treatments were highly significant at $P < 0.01$.

Methods. Leaf disks (10 mm in diameter) were taken 3.5 h after the ozone was turned on and incubated in 3.5 ml sodium phosphate buffer (10 mM, pH 7.0) at room temperature and a light fluence of 300 µmol m⁻² s⁻¹ for 24 h. Ethylene and ethane emissions from these disks were analysed on a Pye Unicam 104 in a GLC fitted with a 1-m Poropak Q 80/100 column and using flame ionization detection with a N₂ carrier gas flow rate of 40 ml min⁻¹ at 100 °C. Leaf injury was scored 48 h after the plants were taken out of the cabinets by assessing the visible leaf injury on all leaves without the scorer having prior knowledge of what the treatment had been. All experiments were repeated three times with 7 plants per treatment each time.

* No visible injury; † 7 h every day; ‡ also with raised rates of ethane evolution.

the extent of visible leaf injury caused by ozone. To test this hypothesis, three-week-old clean-air-grown pea seedlings were pretreated with an inhibitor of ethylene biosynthesis, aminoethoxyvinylglycine (AVG) before they were exposed to 7 h of 150 p.p.b.v. ozone. AVG is known to inhibit the enzyme, 1-aminocyclopropane-1-carboxylic acid (ACC) synthase during the biosynthesis of ethylene⁷. We reasoned that if ethylene evolution is responsible for ozone injury then AVG should offer some protection. Consequently, pea plants were sprayed twice until run-off with AVG (1 mM AVG, containing 0.05% Tween 20) on the day preceding the ozone treatment. This inhibitor treatment beforehand reduced ethylene production by 85% during exposure to ozone and also almost abolished the visible leaf injury normally caused by this short ozone fumigation (both $P < 0.01$; Table 2), confirming our prediction.

In the environment, ozone rarely occurs in isolation but often together with other atmospheric pollutants such as nitrogen dioxide and nitric oxide. Accordingly, we investigated if such additional air pollutants increased ozone-mediated leaf injury by increasing stress ethylene production. When three-week-old clean-air-grown pea seedlings were fumigated with different concentrations of ozone and NO and/or NO₂, significant increases in both stress ethylene ($P < 0.01$) production and in visible leaf injury ($P < 0.05$) were found (Table 3). In addition to greater emissions of ethylene, higher rates of ethane formation were also found. These are indicative of a secondary phase of leaf injury⁸ and were increased in those plants which showed severe visible leaf injury (Table 3). However, fumigation with either NO or NO₂ alone failed to cause any visible leaf injury although they increased ethylene (but not ethane) production

Table 2 Ethylene production and leaf injury in AVG pretreated pea seedlings

Treatment	C ₂ H ₄ evolved (nmol per g dry weight per h)	Visible leaf injury (%)
Control	2.6 (±0.4)	>50
10 ⁻³ M AVG	0.4 (±0.1)*	0–5*

* $P < 0.01$.

Table 3 Leaf injury and formation of ethane and ethylene after mixed fumigations of 3-week-old, clean-air-grown pea seedlings

Fumigation treatment	C ₂ H ₄ evolved (nmol per g fresh weight per h)	C ₂ H ₆ evolved (nmol per g fresh weight per h)	Visible leaf injury (%)
Controls	2.6 (±0.2)	0.5 (±0.1)	—
150 p.p.b.v. NO	6.5 (±1.4)	0.4 (±0.1)	none
150 p.p.b.v. NO ₂	8.2 (±1.3)	0.5 (±0.1)	none
Ozone (50 p.p.b.v.) plus			
50 p.p.b.v. NO	2.3 (±0.3)	0.5 (±0.1)	0–5
100 p.p.b.v. NO	4.1 (±0.4)	0.5 (±0.1)	15
150 p.p.b.v. NO	3.3 (±0.3)	1.3 (±0.2)	20*
Ozone (100 p.p.b.v.) plus			
50 p.p.b.v. NO	2.5 (±0.3)	1.3 (±0.2)	40
100 p.p.b.v. NO	3.9 (±0.2)	1.2 (±0.2)	60*
150 p.p.b.v. NO	3.7 (±0.5)	1.5 (±0.2)	90†
Ozone (100 p.p.b.v.) plus			
50 p.p.b.v. NO ₂	2.0 (±0.4)	1.3 (±0.2)	40
100 p.p.b.v. NO ₂	3.4 (±0.2)	1.2 (±0.2)	50*
150 p.p.b.v. NO ₂	3.3 (±0.3)	1.4 (±0.2)	60*

* $P < 0.05$; † $P < 0.01$. (Obtained when combined treatments were compared to the ozone treatment alone.)

significantly ($P < 0.01$). It does, however, predispose them to subsequent ozone injury (data not shown).

Reaction processes involving ozone within tissues are complex but may involve either ozonolytic or peroxidative processes⁹. Both produce malonaldehyde but the latter are distinguished by being initiated by free radical attack. At low levels of ozone, peroxidation would be favoured over ozonolysis by the low solubility of ozone¹⁰ in the aqueous phase of the cell. Furthermore, reaction of ozone with small unsaturated hydrocarbons such as ethylene is known to produce a number of water-soluble, highly reactive free radicals¹¹. These may initiate peroxidative events inside plant cells that could ultimately cause macroscopic leaf necrosis. It is possible that the mechanism for ozone toxicity

presented here may also have implications for animals, where ethylene may be formed as a breakdown product during polyunsaturated fatty acid oxidation^{12,13}.

Our results indicate that intermittent episodes of elevated levels of atmospheric ozone may be more phytotoxic to peas than constant high levels of ozone because there is greater tolerance to ozone when rates of ethylene formation are depressed. The presence of additional pollutants such as the oxides of nitrogen significantly enhances ozone-mediated leaf injury by increasing stress ethylene production. These results imply that, when agricultural plants in the field experience other stresses, such as chilling which also produce stress ethylene, plant sensitivity to ozone may be much higher than has previously been assumed. Furthermore, these results may be highly relevant to an understanding of recent forest decline, which is especially pronounced at higher altitudes where ozone levels may often be higher and more persistent but where natural environmental stresses or mineral leaching due to acidic precipitation are often greatest.

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Environmental consequences of treating cattle with the antiparasitic drug ivermectin

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Ivermectin (22,23-dihydroavermectin B₁) is a recently discovered, persistent, broad-spectrum, antiparasitic drug of unprecedented potency^{1,2} which is now routinely administered to cattle, horses, sheep and pigs in many countries. In cattle, it is an efficient control for parasitic gastrointestinal and respiratory tract nematodes, warble fly, mites, lice and ticks^{3–6}. However, most of the ivermectin dose is ultimately eliminated in the faeces of the treated animals⁷ where it has been shown to have an insecticidal effect^{8–11} on the larvae of economically important, dung-breeding, haematophagous Diptera. Nevertheless, the effects of excreted ivermectin on the cowpat fauna as a whole and the wider consequences of such effects have not previously been considered. In field trials reported here, the faeces of calves fitted with rumenal boluses delivering ivermectin at 40 µg per kg per day, failed to degrade in the normal way and this failure was associated with the absence of dung-degrading insects. Faeces from placebo-treated controls contained a characteristic dung-degrading invertebrate community and were largely degraded within 100 days. These results indicate that the increasing widespread use of ivermectin may have important environmental consequences for pastureland.

Two groups of four Friesian calves (200 kg) were maintained under identical conditions. The experimental group was dosed on 3 June 1986 with ivermectin (Merck, Sharpe and Dohme) in the form of a bolus implanted in the rumen. The bolus provided a constant release of ivermectin at a concentration of 40 µg per kg per day. The calves in the second group were placebo-treated and acted as controls. The collection of fresh dung (0–12 h old) was started 11 days after the implantation of the ivermectin boluses and continued throughout six subsequent days. The dung obtained at each collection was mixed thoroughly for each group, before immediate use. The field site was a 5 m × 10 m enclosure in a pasture used for dairy cattle and some sheep grazing (Wyndhurst Farm, University of Bristol). Circular formers of corrugated cardboard (0.3 m diameter, 0.03 m depth¹²) were each filled with 2,000 g of dung, weighed out on a hand-held, spring balance. Control and experimental pats were evenly spaced at 1 m intervals in an alternating sequence across the enclosure. Grass surrounding the pats was cropped to 10–15 cm throughout the experiment to control for possible microclimate differences. Two control and two experimental pats were covered by cages 10 days after they were prepared. Comparison of caged and uncaged pats showed that small mammals and birds were not an important source of pat degradation in these experiments.

Control and experimental pats were collected after 20, 30, 40, 50, 60, 80 and 100 days. The soil immediately beneath each pat was also removed to a depth of about 2 cm. Pat and soil samples were searched for invertebrates, which were identified and counted. A characteristic invertebrate community¹³ was present in the control pats and soil. Coleoptera were abundant (Table 1); of these 89.4% were Scarabaeidae of primarily *Aphodius*

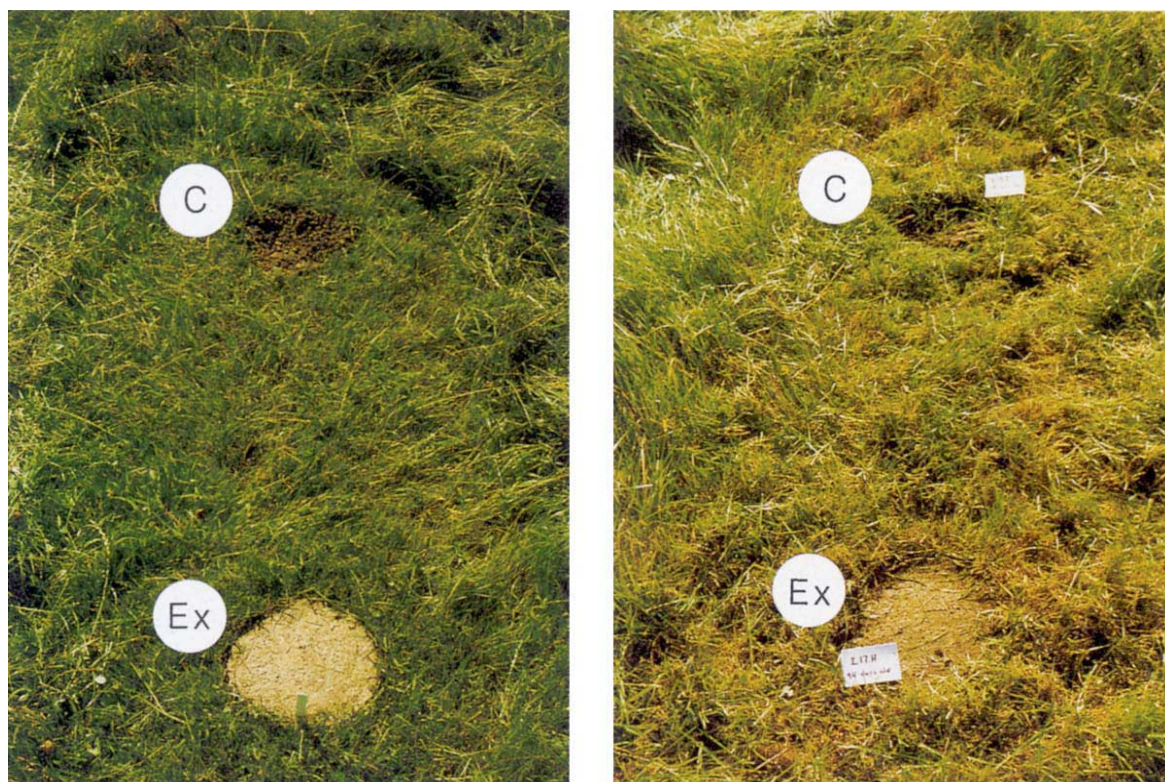


Fig. 1 Control (C) and experimental (Ex) pats at *a*, 40 days and *b*, 94 days after deposition.

spp. The families Geotrupidae, Staphylinidae and Carabidae accounted for 4.7%, 3.1% and 1.3% of the beetles respectively. Small numbers of Hydrophilidae and Elateridae were also present. The data (Table 1) illustrate a clear pattern of initial beetle colonization, larval growth, pupation and adult emergence over the first 50 days or so of the life of the pats. The second most abundant insect order was the Diptera: Bibionidae, Stratiomyidae and Chironomidae were the most common families representing 56.6%, 18.6% and 11.8% of the larvae respectively, and small numbers of Mycetophilidae, Muscidae, Sepsidae and Syrphidae were also present. Puparia were not identified. Earthworms were initially found only in the soil beneath the pats, but from 40 days onwards were present in both pats and soil. The category 'other' invertebrates mainly consisted of centipedes. In contrast, the experimental pats and soil were almost sterile containing few, or in some cases no

Coleoptera or Diptera (Table 2). There was some indication that dipteran pupae were able to survive in the soil from about 60 days onwards. Earthworms were present in the soil under the experimental pats, but only in the 80- and 100-day samples were they found consistently in the pats themselves. The category 'other' invertebrates mainly consisted of centipedes.

In the control pats, the timing of beetle larval activity, pupation and emergence coincided with the rapid break-down of pat structure (Fig. 1*a*). In the experimental pats no such decomposition occurred and at 40-50 days they were as intact as when first prepared (Fig. 1*a*). Subsequent decomposition of the control pats was less dramatic, but nevertheless by 100 days they had largely disappeared (Fig. 1*b*). However, even at 100 days the experimental pats were still largely intact, retaining a solid crust and showing signs of erosion only at the margin (Fig. 1*b*). A quantitative estimate of the difference in the rates of decompo-

Table 1 Invertebrates in control pats and soil samples

		No. of samples	Coleoptera			Diptera		Earthworms	Other
			L	P	A	L	P		
20 days	Pat	2	474	0	6	3	3	0	0
	Soil	2	4	3	1	2	3	1	0
30 days	Pat	2	171	4	0	18	2	0	0
	Soil	2	31	4	2	6	0	7	4
40 days	Pat	4	68	281	7	71	3	2	0
	Soil	3	4	7	1	19	1	7	0
50 days	Pat	2	58	37	92	22	1	3	2
	Soil	2	3	0	0	1	75	5	1
60 days	Pat	1	8	3	3	127	3	7	2
	Soil	1	1	0	0	176	0	9	0
80 days	Pat	2	1	1	6	20	2	23	3
	Soil	2	1	4	4	8	16	35	0
100 days	Pat	3	0	0	9	6	5	1	0
	Soil	3	0	2	8	5	16	18	3
Total	Pat	16	780	326	123	267	19	36	7
	Soil	15	44	20	16	217	111	82	8

The number of larval (L), pupal (P) and adult (A) Coleoptera, larval (L) and pupal (P) Diptera, earthworms and 'other' invertebrates are shown.

Table 2 Invertebrates in experimental pats and soil samples

		No. of samples	Coleoptera			Diptera		Earthworms	Other
			L	P	A	L	P		
20 days	Pat	2	9	0	2	0	0	0	0
	Soil	2	4	0	6	0	3	1	0
30 days	Pat	2	0	0	0	0	0	2	0
	Soil	2	0	0	0	0	4	10	0
40 days	Pat	2	0	0	0	0	0	0	0
	Soil	2	0	0	0	0	0	8	0
50 days	Pat	2	1	0	0	1	0	0	2
	Soil	2	0	1	4	2	2	16	1
60 days	Pat	1	0	0	0	0	0	0	0
	Soil	1	0	0	0	0	21	8	0
80 days	Pat	2	1	1	4	1	2	15	1
	Soil	2	0	0	2	2	18	25	0
100 days	Pat	6	6	0	5	33	3	27	5
	Soil	6	2	0	5	0	37	33	3
Total	Pat	17	17	1	11	35	5	44	8
	Soil	17	6	1	17	4	85	101	4

Abbreviations as in Table 1.

sition of control and experimental pats is presented in Fig. 2.

The insecticidal effects of ivermectin excreted in the faeces of cattle treated with a variety of delivery systems have been examined previously. Oral capsules delivering 1, 5, 10, 50, 100 and 200 μg per kg per day, subcutaneous injections of 5, 10 μg per kg per day, a single injection of 200 μg per kg, rumenal boluses delivering about 50 μg per kg per day and rumenal tablets delivering about 50 μg per kg per day are all lethal to *Haematobia irritans* and doses of 20 μg per kg per day, or above, are 100% effective against *Stomoxys calcitrans*⁸. At present, the routine treatment of cattle is by one, or repeated subcutaneous injections, at the recommended dose of 200 μg per kg. A rumenal bolus or similar delivery system is likely to have a more pronounced insecticidal effect in dung than injection because the bolus may remain active for many months releasing much of its ivermectin directly into the gut and into the faeces⁸. Nevertheless, enough ivermectin is excreted following a single injection of 200 μg per kg to eliminate non-pest Diptera (such as Sphaeroceridae and Sepsidae) as well as *H. irritans* in dung, for at least 35 days following treatment⁹. Such studies have been concerned with the use of excreted ivermectin for controlling dung-breeding pests and, therefore, have focused on a narrow range of dipteran species. This field trial shows that excreted ivermectin in the faeces of calves treated with a rumenal bolus delivering 40 μg per kg per day, has an insecticidal effect on the entire dung-degrading insect community.

Some caution must be exercised in drawing general conclusions about the use of ivermectin from results obtained under the specific conditions used in this study, because the severity of such effects is likely to be related to variation in the dose administered, the delivery system used and perhaps even the climate, geographical area and insect species present. Nevertheless, our results confirm and extend the implications of previous work⁸⁻¹¹, in indicating that the increasing routine use of ivermectin in cattle is likely to pose a serious ecological threat to those insects (particularly Diptera) that have evolved to exist uniquely in conjunction with cattle dung. Ivermectin is also available for the treatment of horses and sheep, bringing a similar threat to their dung communities. In addition, in this study, the absence of dung-degrading insects, particularly beetles, in the faeces of the treated calves was associated with the failure of pats to degrade in the normal way. Retarding pat decomposition may increase the amount of pasture fouled by cattle dung and, therefore, could have important consequences for grassland husbandry¹⁴⁻¹⁶. The elimination of dung beetles could also have particularly far-reaching consequences for the ecology and pasture management of Australia. The droppings of introduced

cattle and horses are completely different from those of the principal marsupial herbivores and cannot be disposed of by the native Australian dung beetles. This resulted in extensive fouling of valuable pasture land and led to the initiation of the 'Dung Beetle Project' by CSIRO in 1964, in which African dung beetles were imported, bred and released¹⁷. The continued success of this and similar schemes¹⁸ could be seriously affected by the insecticidal effects of avermectins. The results of this field trial, therefore, highlight a previously ignored area of serious concern and indicate the urgent requirement for further work to clarify this situation.

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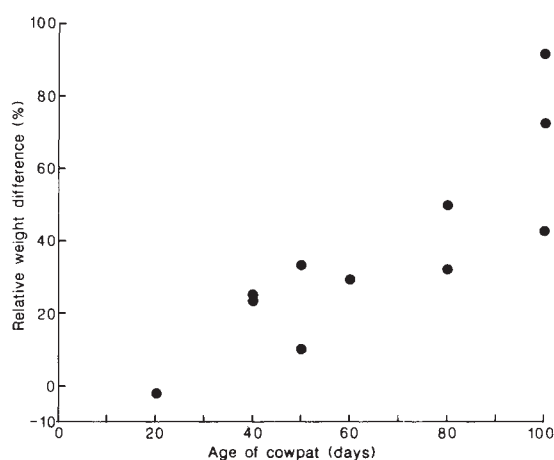


Fig. 2 Difference in weight of control and experimental pats. At each collection the wet weights of pats sampled were recorded. At least one control and one experimental pat in each age class were sampled on the same day. The wet-weights of control and experimental pats, of the same age, sampled on the same day, may be used to obtain a relative measure of weight difference, free from the effects of variation in rainfall. This is achieved by expressing the absolute wet-weight difference between the control and experimental pats of a pair, sampled at the same time as a percentage of the wet weight of the experimental pat (relative weight difference). At 20 days, the control was slightly heavier than the experimental pat as consequence of the large numbers of beetle larvae in the control and the relative sterility of the experimental pats at this age. In all subsequent samples the control pats were lighter than the experimental pats and this difference progressively increases as the control pats decompose more rapidly than the experimental pats.

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Embryonic and adult neurons interact to allow Purkinje cell replacement in mutant cerebellum

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It has often been proposed that one way of replacing degenerating neurons in the brain is to implant embryonic neurons of the same type¹. However, in the case of so-called 'point-to-point' systems, as opposed to the 'paracrine' systems which mainly involve local release of neurotransmitter, functional recovery requires a precise re-establishment of the missing circuitry. We recently showed that in one point-to-point system, the cerebellum of adult mice homozygous for the mutation *Purkinje cell degeneration (pcd)*², missing Purkinje cells can be replaced by grafting cerebellar primordia from normal mouse embryos^{3,4}. Here, we present studies of the cellular mechanisms underlying this successful replacement. Grafted Purkinje cells leave the graft to migrate along stereotyped pathways to their final position in the deficient molecular layer, where they receive synaptic contacts from adult host neurons. Both the detailed timetable and the precise cellular interactions observed are remarkably similar to those occurring during normal development. Our results suggest that the deficient molecular layer exerts a selective neurotropic effect on neurons of the missing category, and that the embryonic neurons are able to respond to this signal during a period defined by their own internal clock. We also raise the possibility that embryonic Purkinje cells can induce in adult neural cells a new type of plasticity, that of recreating a permissive microenvironment for the integration of embryonic neurons.

Purkinje cell degeneration (*pcd*) is a recessive mutation which arose spontaneously in the C57BL/cdJ mouse strain². The mutant *pcd* allele intrinsically leads to the death of cerebellar Purkinje cells⁵, a process which is almost complete two months after birth^{2,6}. For our grafting experiments, we used as hosts three- to four-month-old homozygous *pcd* mice (raised at the Pasteur Institute by Dr J. L. Guénet). In all cases, donor tissue was taken from isogenic cerebellar primordia of 12-day-old (E12) embryos. In each mutant cerebellum, two grafts symmetrically positioned at the borders between the vermis and hemispheres were analysed 4 to 21 days after transplantation. The fate of grafted Purkinje cells was detected immunohistochemically using an antiserum against calbindin, a vitamin D-dependent calcium-binding protein^{7,8} of relative molecular mass 28,000 (*M_r* 28K) which in cerebellum is exclusively located in

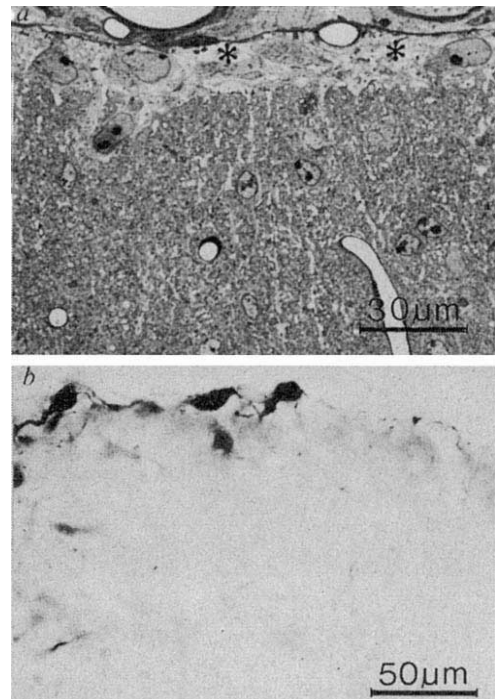


Fig. 1 Grafted Purkinje cells five days after transplantation. *a*, Toluidine-blue stained section and *b* anti-calbindin stained section. At this stage (*a*), a migratory cellular stream occupies a newly formed region (asterisks) between the pial basal lamina and the glial limiting membrane. The most distal part of the migratory stream consists of a single row of cells containing large cells with elongated perikarya (about 20 μm in their largest diameter). No mitotic figures were observed among the migrating neurons and all of them (*b*) are calbindin-immunoreactive. Therefore, they must have been young postmitotic Purkinje cells, with bipolar configuration, which expand parallel to the cerebellar surface (phase of horizontal polarity and tangential migration).

Methods. *a*, the brain was prepared for conventional electron microscopy by perfusing with 1% glutaraldehyde and 1% paraformaldehyde in 0.12 M phosphate buffer pH 7.4, followed by immersion-fixation in 2% osmic acid in the same buffer, and Araldite embedding. The micrograph was obtained from a 1-μm thick plastic section, cut in the sagittal plane, and stained with toluidine blue. In *b*, the brain was prepared for calbindin-immunohistochemistry by perfusing with 4% paraformaldehyde in 0.12 M phosphate buffer, pH 7.2. Frozen sections were cut (20 μm) in the sagittal plane and incubated in 1:8,000 anti-calbindin²¹. Specific antibody binding was visualized using peroxidase-anti-peroxidase²².

Purkinje cells^{9,10} and expressed early in development¹⁰.

Implants integrated with the host cerebellum at four days after grafting. Immediately afterwards, calbindin-immunoreactive cells moved away from the grafts into the host cerebellum. By five days, a funnelling migratory stream had formed at the folial surface, between the subpial basal lamina and the molecular layer, with its wide end at the host-graft interface. The calbindin staining (Fig. 1a) and the absence of mitotic figures (Fig. 1b) in the moving cells indicated that only postmitotic Purkinje cells were involved in the tangential migration. No migration was observed when implants were grafted in normal mouse cerebellum (data not shown). These observations strongly suggest that the Purkinje-deficient molecular layer exerts a positive neurotropic effect¹¹, selective for neurons of the same category as those missing, which is suppressed when Purkinje cells are present, as in the normal mouse.

Six to seven days after transplantation, the tangential migratory stream attained its maximal extension, covering about 700 μm on either side of the graft-host borders. At the same time, Purkinje cells massively penetrated the molecular layer.

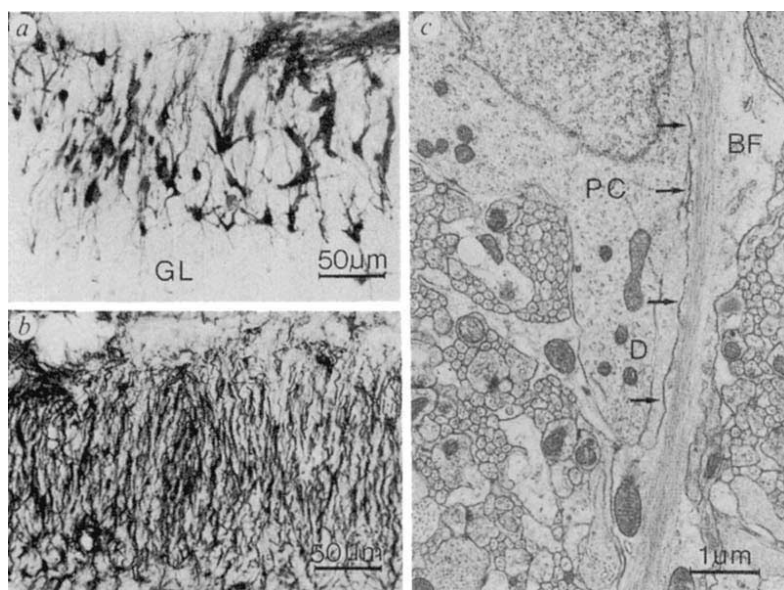
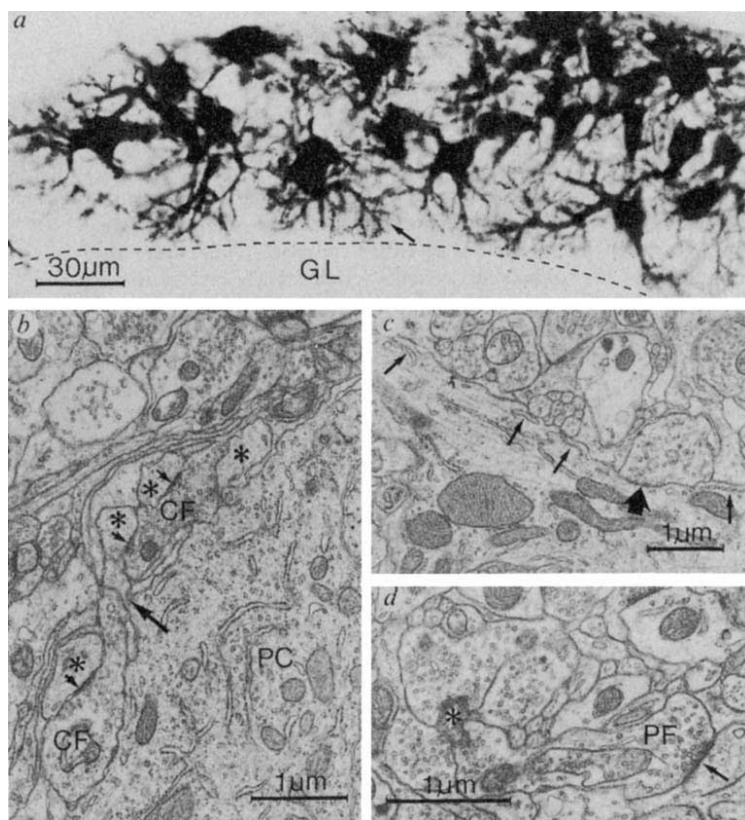


Fig. 2 Grafted Purkinje cells seven days after transplantation. At this stage (a), most of the migrating calbindin-immunoreactive neurons have changed polarity and exhibit a radial orientation, spanning the whole depth of the host molecular layer. Note that the inward growing processes stop at the upper limit of the granular layer (GL), and that a few neurons lie in an oblique or even horizontal position. In a succeeding section of the same cerebellum (b), Bergmann fibres, stained with antisera against vimentin, maintain their palisade arrangement. c, The ultrastructural features of a radial migrating Purkinje cell. The cytoplasm contains abundant polyribosomes suspended in a light cytoplasmic matrix but very few, short cisterns of the rough endoplasmic reticulum, as in immature Purkinje cells¹⁰. Note the absence of synaptic contacts on the cell body (PC), and on the inward growing dendrite (D). More importantly, the migrating neuron is directly apposed (arrows) to a Bergmann fibre, suggesting that specific neuro-glial interactions regulate the radial migration of at least some grafted Purkinje cells.

Methods. Material in a and c was processed as in Fig. 1 legend. Immunohistochemical staining of the Bergmann fibres was performed using a dilution of 1:1,000 of anti-vimentin²³, following the protocol in Fig. 1 legend.

Fig. 3 Grafted Purkinje cells 15 days after transplantation: At this stage, the cell bodies of calbindin-immunoreactive neurons (a) remain confined to the host molecular layer, without penetrating the granular layer (GL), as in the previous stage. However, the dendritic trees have dramatically changed. Now they span the molecular layer and extend their arbors in the sagittal plane. Note the abundant spines (arrow) emerging from distal spiny branchlets. The synaptic investment of the grafted neurons, illustrated in the electronmicrographs in c-d, indicates that synaptogenesis is very active. b, Purkinje cell body (PC) with a much more mature appearance than 7 days after grafting (compare with Fig. 2c). Its cytoplasm contains parallel arrays of rough endoplasmic reticulum and beginnings of the formation of Nissl bodies. Long spine-like filopodia emerge from the soma (large arrow). They are synaptically contacted (small arrows) by axonal varicosities, filled with numerous spherical vesicles, belonging to the host climbing fibre (CF) system. This pseudoglomerular synaptic arrangement corresponds to the 'pericellular nest stage' in climbing fibre/Purkinje cell synaptogenesis¹⁷. c, Profiles identified as Purkinje cell proximal dendrites, by the constant presence of hypolemmal cistern²⁴ (small arrows), are occasionally postsynaptic to medium-sized boutons, containing pleomorphic synaptic vesicles, and establishing symmetrical synaptic contacts (large arrow). The morphology of these boutons allows their identification as belonging to ascending collaterals of the basket cell axons or to axon terminals of stellate cells. d, Dendritic spine, belonging to a distal spiny branchlet of a grafted Purkinje cell, synaptically contacted (arrow) by a parallel fibre varicosity (PF). Note that the latter still maintains a pseudo-synaptic contact with a necrotic debris of a pcd Purkinje cell dendrite (asterisk).



The majority of these neurons changed polarity and adopted a bipolar radial disposition (Fig. 2a). Inward-oriented processes penetrated the whole depth of the molecular layer, but did not enter the granular layer, as if their permissive environment abruptly stops at the molecular layer/granular layer interface. This phase of radial migration mimics that taking place in normal ontogeny (Fig. 2a), with one essential difference: it occurs in an opposite direction, migration in development being from the ventricular primitive neuroepithelium towards the cerebellar surface^{12,13}. The close parallelism between some of the radially-oriented Purkinje cells and the Bergmann fibre palisades raises the possibility of glial guidance of their migration¹⁴ (compare

Fig. 2A and B). Direct evidence for such neuronal-glial interaction was obtained ultrastructurally, because in random sections, some of the migrating Purkinje cells had their perikarya and lower processes directly apposed to Bergmann fibres (Fig. 2c). At this stage, intense synaptogenesis had not started and the cell bodies and dendritic processes of the Purkinje cells were almost free of synaptic contacts (Fig. 2c).

Fifteen days after transplantation, in regions of the pcd cerebellum containing grafted neurons the molecular layer was spanned by rows of Purkinje cells, still distributed in the superficial four-fifths (Fig. 3a). This unchanged disposition confirms that the arrest of migration and perikaryal translocation

Table 1 Purkinje cell development in grafts

Morphological events	Age of Purkinje cells	
	Normal ontogeny*	Grafts
Initiation of migration	E14	4DAG (E16)
Arrest of migration	E17-E19	7DAG (E19)
Acquisition of a monopolar dendritic tree	End 1st postnatal week	15DAG (P7)
Early stage in climbing fibre synaptogenesis 'pericellular nest stage'	P6-P9	15DAG (P7)
Initiation of parallel fibre-Purkinje cell synaptogenesis	P6	15DAG (P7)
Initiation of perikaryal investment by basket fibres	P7	15DAG (P7)
Initiation of dendritic investment by basket and stellate axons	P8	15DAG (P7)

* Data from refs 10, 12, 17, 19 and 20.

DAG, days after grafting; E, embryonic day; P, postnatal day.

is concurrent with the arrival of the inward-growing processes at the upper limit of the granular layer, seven days after grafting. This arrest also explains the ectopic locations of grafted Purkinje cell bodies in long-survival transplants^{3,4}.

At the end of the migratory period, grafted Purkinje cells began to develop the final form of their dendritic trees. By 15 days after implantation, they had acquired two features which characterize normal Purkinje cell dendrites¹⁵: a monopolar disposition in a plane perpendicular to the bundles of parallel fibres running through the host molecular layer and a spiny appearance (Fig. 3a). However, owing to the ectopic location of their perikarya, some of these Purkinje cells have inverted dendrites, whereas others are multipolar (Fig. 3a).

At this stage, synaptogenesis between axons belonging to the adult host brain and grafted Purkinje cells was very active. The climbing fibres formed 'pericellular nests'¹⁶, establishing synaptic contacts on somatic filopodia (Fig. 3b). Axon terminals of basket and stellate cells synapsed on the smooth surface of either the perikarya or the thick proximal dendrites (Fig. 3c). Numerous varicosities of parallel fibres synapsed on the still immaturely enlarged spines of the distal spiny branchlets. The occasional presence of parallel fibre axon terminals attached to necrotic debris, reminiscent of the degenerated pcd Purkinje cell dendrites³ and synapsing on newly formed spines (Fig. 3d), indicates that the parallel fibres involved in the synaptogenesis were those of the mutant molecular layer.

Twenty-one days after transplantation, most of the developmental processes had reached an end. The grafted Purkinje cells exhibited dendritic trees and had acquired a synaptic investment qualitatively similar to those reported in long-term survivals^{3,4}.

The results reveal a striking similarity in timing for the developmental events leading to the maturation of Purkinje cells during normal ontogeny and in the adult pcd cerebellum (Table 1). Purkinje cell proliferation lasts from E11 to E13 in the embryo¹². Preliminary autoradiographic experiments with ³H-thymidine show that injection of the isotope into host adult mice labels grafted Purkinje cells only until one day after grafting (E13). In normal ontogeny, Purkinje cells begin to migrate shortly after the end of their proliferative period¹², and depending on the cerebellar region¹³, attain their cortical location between E17 and E19¹⁰. Migration in the grafts, which cannot start before transplant integration, is finished seven days after implantation (E19). Synaptogenesis and segregation of inputs in the grafted Purkinje cells also proceeds following a schedule very close to that in ontogeny. The most remarkable sign of this correspondence is the presence of synapses on both filopodia and smooth regions of the soma of Purkinje cells, 15 days after grafting. In normal ontogeny, axo-somatic synapses on filopodial and smooth regions characterize a short develop-

mental period marked by the end of the transient 'pericellular nests' of climbing fibres and the beginning of the final basket investment. This phase takes place in normal mouse development toward the end of the first postnatal week¹⁷, the real age of the Purkinje cells 15 days after transplantation. These results indicate that the grafted Purkinje cells influence target-deprived axons of the deficient molecular layer to initiate synaptogenesis, and that these immature cells impose on sprouting adult axons a timed sequence of maturation corresponding to that occurring normally in ontogeny.

Grafted Purkinje cells thus follow a predetermined pattern of maturation as if an internal clock, independent of environmental signals, regulated their developmental programme. This could explain the limited outgrowth of grafted Purkinje cells into the deficient pcd molecular layer in spite of the constant presence of Purkinje cells in the implant remnants^{3,4}. The migratory behaviour of Purkinje cells lasts, in the embryo, for a maximum of six days after final division¹³, equivalent to seven days after grafting in our experimental conditions, and it is at this time that the invasion of the molecular layer is maximal (700 μ m). A similar constraint in the time window for migration has been reported for cerebellar granule cells¹⁸. Thus, as grafted cells mature, they must lose their ability to migrate despite the continuing attraction of the deficient molecular layer.

In spite of the time mismatch, embryonic Purkinje cells grafted into adult pcd cerebellum do migrate to their proper domains, develop dendritic trees, and integrate synaptically into the deficient circuitry of the cerebellar cortex. These results indicate that signals for neurotropism, migration, neuronal differentiation and synaptogenesis are not limited to embryos but can be expressed in the adult, at least in pcd mice. This might mean that the mutant cerebellum can express these immature signals throughout adult life, owing either to the mutation itself or to the lack of the major cortical target, the Purkinje cells. A more interesting possibility is that embryonic Purkinje cells by themselves regulate gene expression of adult neurons and glial cells by generating a transient permissive microenvironment, thus allowing synaptic integration of the grafted neurons, leading to the subsequent restoration of the impaired cortico-cerebellar circuitry.

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Signal requirements in the step-wise functional maturation of cytotoxic T lymphocytes

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The generation of effector cytotoxic T lymphocytes from resting precursors proceeds through a series of steps in a pathway that, in aggregate, involves both proliferation and development of cytotoxicity¹⁻⁷. To understand the relationship of the various signals (mitogens and/or lymphokines) that bring about progress along this pathway, it is desirable to define a series of 'minimal signals', each of which stimulates the cell to proceed to a further stage in this process of differentiation. Stimulation of lymphocytes with two different monoclonal antibodies directed against the CD2 surface molecule induces a proliferative response⁸; we report here that both CD4⁺ and CD8⁺ cells proliferate in response to such a stimulus but do not develop cytotoxicity. Addition of recombinant γ -interferon (rIFN- γ) or recombinant IL-2 (rIL-2) to the activated cells leads to acquisition of cytotoxic status by the CD8⁺ cells but not the CD4⁺ cells. The availability, in addition to precursors and effectors, of an apparently intermediate stage in the form of proliferating CD8⁺ cells that are non-cytotoxic should facilitate both cellular and molecular studies of this maturation pathway; the differences between CD4⁺ and CD8⁺ cells in development of cytotoxicity under these experimental conditions is a valuable model for understanding differentiation of T lymphocytes.

The concept that more than one signal is required to drive resting precursors to the proliferating and functionally active effector stage, and that individual minimal signals may drive cells only part of the way along the pathway of functional maturation, has existed for more than ten years¹⁻⁷. Initial models were based on similar designs for B cells^{9,10}.

Peripheral blood lymphocytes (PBL) were negatively selected by panning and studied as outlined in Fig. 1. Negative selection was used to retain adherent cells in culture and yet allow very significant enrichment for either CD4⁺ or CD8⁺ cells. Cells of these three populations (CD4⁺, CD8⁺ and unpanned PBL) were individually cultured either without stimulation, or in the presence of two monoclonal antibodies (mAbs), VIT13 (ref. 11) and 9.6 (ref. 12).

This combination, as well as each of rIL-2 and OKT3 were highly effective at stimulating proliferation in CD8⁺ cells (see Fig. 2a). In parallel with the proliferative response, each of these three stimuli led to up-regulation of the IL-2 receptor as measured with the Tac mAb (Fig. 2b). However, stimulation with the two mAbs against CD2, but not with OKT3, was not accompanied by an increase in detectable IL-2 production (Fig. 2d). Furthermore, antibody against Tac was not able to block proliferation induced by VIT13 and 9.6 whereas OKT3-driven proliferation was inhibited significantly (data not shown). These findings suggest that cells stimulated with VIT13 and 9.6 proliferate in a relatively IL-2-independent manner. Stimulation with IL-2 and OKT3 led to very significant levels of cytotoxicity. However, stimulation with only the mAbs against CD2 did not lead to development of cytotoxicity on day 4 (Fig. 2c). Representative results for CD8⁺ cells are shown but PBL and CD4⁺ cells respond in a similar way: CD4⁺ cells stimulated with the mAb combination did not produce detectable levels of IL-2 (data not shown). These findings encouraged us to evaluate VIT13 and 9.6 as a possible stimulus to generate IL2R⁺, proliferating, but non-cytotoxic pre-effector T lymphocytes.

Following four days of incubation with VIT13 and 9.6, the responding blasts were washed and positively sorted on a fluorescence-activated cell sorter (FACS). Responding blasts were negative in a lectin-dependent cytotoxic assay (LDCC) before and after FACS sorting (data not shown). The blasts were incubated for an additional one, two, or three days with either tissue culture media (TCM), rIFN- γ or rIL-2. The cells were assayed for both proliferation and cytotoxicity (in an LDCC assay) after these extra days of incubation.

Highly significant levels of cytotoxicity were detected in PBL and CD8⁺ cells stimulated for 3 or 4 days with VIT13 and 9.6 followed by 2 or 3 days stimulation with rIFN- γ or rIL-2, that is as early as 5 days after the start of the culture (Fig. 3). The need for two signals to generate cytotoxicity is further supported by the observations that PBL or CD8⁺ cells stimulated with mAbs against CD2 for as long as 6 days, or CD8⁺ cells stimulated with VIT13 and 9.6 for 4 days and then with TCM for an additional 3 days, both failed to develop cytotoxicity (see Fig. 3). Furthermore, CD4⁺ cells do not develop cytolytic function as late as day 7, whether or not rIFN- γ or rIL-2 is present during the second incubation. It has been reported that CD8⁺ T-lymphocyte clones can be driven to kill a non-related target by mAb against CD2 (ref. 13); such cloned effectors are already programmed for cytotoxicity before stimulation with antibody against CD2 and thus represent a different system from the one studied here.

Both rIFN- γ and rIL-2 when added to the CD8⁺ cells allow them to become cytotoxic; rIFN- γ does not stimulate further proliferation, whereas rIL-2 does. Whether rIL-2 works directly or indirectly by stimulating production of another lymphokine, such as IFN- γ , that in turn is important for the maturation process, is not known.

In our studies, the levels of cytotoxicity seen in the CD4⁺ cells, even after stimulation with rIFN- γ or rIL-2, are close to the background levels of cytotoxicity (see Fig. 3). The finding that, under the experimental conditions used, CD4⁺ cells did not develop lytic function is intriguing. We wonder whether there is pre-commitment of CD4⁺ and CD8⁺ precursor cells such that CD2 stimulation together with either rIL-2 or rIFN- γ leads to development of cytotoxicity in one population and not the other.

It has been previously shown that IFN- γ leads to acquisition of antigen-specific cytotoxicity by a cloned 'T lymphocyte'¹⁴. Those cells were cultured in IL-2-containing supernatant made with TPA(12-O-tetradecanoyl phorbol-13-acetate), which is known to inhibit cytotoxic function¹⁵; further, in those clones IL-2 did not provide the same stimulus as IFN- γ .

It has previously been shown that the minimal signal provided by UV-light- or heat-treated allogeneic stimulating cells (perhaps with other signals such as interleukin-1) can stimulate precursors to become poised to receive help, a poised T lymphocyte (poTc), although the responding cells neither proliferate nor become cytotoxic¹⁶. The poised T lymphocyte, which has been stimulated by 'antigen' to up-regulate the IL-2 receptor, is a non-dividing¹⁶, non-cytotoxic cell (ref. 17 and R. Miller, personal communication). Inclusion of rIL-2 with the UV-light-treated allogeneic cells as a stimulus to the responding cells leads not only to up-regulation of IL-2 receptor but also to proliferation and development of cytotoxicity¹⁸. A model for these findings is pictured in the upper line of Fig. 4.

Our results show that the combination of VIT13 and 9.6 stimulates precursors to mature to effectors, and that specific signals can effect this step. The cells shown in the model in Fig. 4 have the following characteristics. The precursor (pTc) is a resting cell thought not to express IL-2 receptor or to do so only at very low levels. The pre-effector Tc (peTc), derived by stimulating the precursor with VIT13 and 9.6 is a dividing, non-cytotoxic cell with up-regulated IL-2 receptor that can be driven to effector status (eTc) by either IL-2 or IFN- γ . We did not purify the precursor to eliminate IL2R⁺ cells; nonetheless,

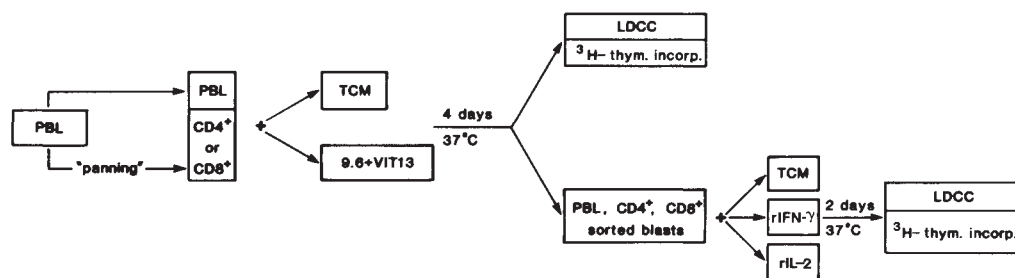
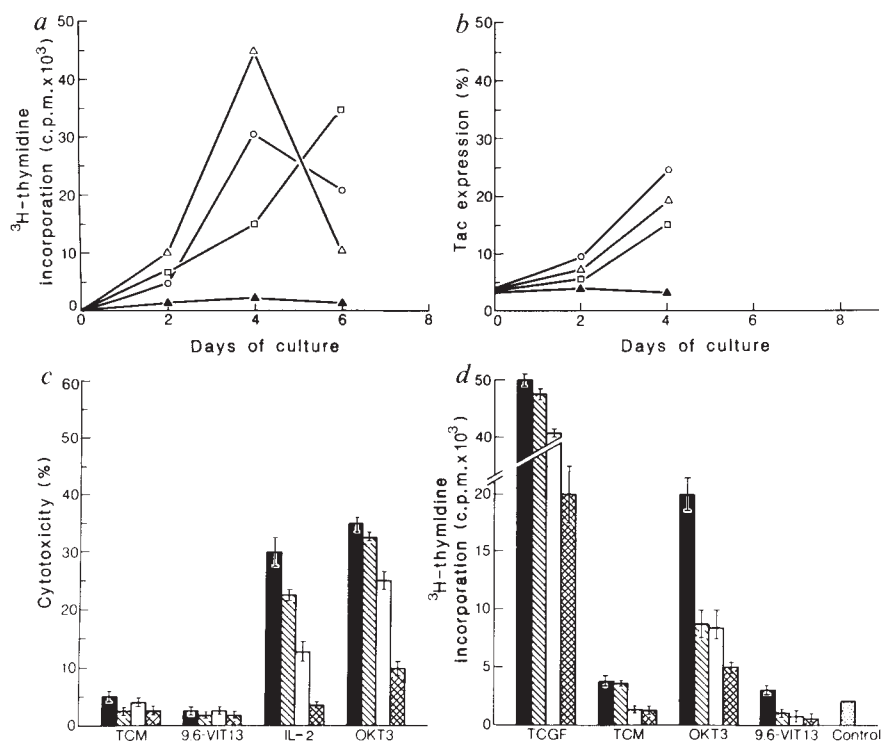


Fig. 1 Experimental scheme. Peripheral blood lymphocytes (PBL), CD4⁺ or CD8⁺ cells were incubated for 4 days in tissue culture medium (TCM) with or without VIT13 and 9.6. On day 4 cells were sorted for size (blasts versus small lymphocytes) and CD phenotype, and returned to culture in TCM or TCM with rIL-2 or rIFN- γ for 2 d. Both ³H-thymidine incorporation and LDCC were assayed before sorting and after the additional 48 h incubation.

Methods. PBL were isolated by Ficoll-Hypaque density gradient centrifugation of heparinized blood from normal healthy donors, washed with phosphate buffered saline (PBS, Gibco) and suspended in TCM consisting of 10% heat-inactivated pooled human serum (HS) in RPMI 1640 containing 25 mM HEPES buffer, supplemented with 2 mM L-glutamine, 100 units ml⁻¹ penicillin and 100 μ g ml⁻¹ streptomycin. CD8 and CD4-enriched fractions were separated by two rounds of adherence of PBLs to antibody-coated plastic petri dishes (panning) as described elsewhere²⁰. Briefly, 30 $\times$ 10⁶ PBL were incubated with OKT8 or OKT4 monoclonal antibody (Ortho), at 1:10,000 dilution at 4°C for 30 min and plated after extensive washing on plastic Petri dishes previously coated with 10 μ g ml⁻¹ of purified goat anti-mouse immunoglobulin G (Ig) (Meloy). Cells were allowed to adhere to the Petri dishes for 70 min at 4°C. The non-adherent population was removed by gently washing the plates with 5% HS-PBS. Viability of the cells after panning was routinely between 95% and 99% as determined by trypan blue dye exclusion. The purity of the cell population was measured by indirect immunofluorescence using a FACS (Becton-Dickinson) (see Fig. 2b legend). CD cross-contamination was between 1% and 2% and remained the same throughout the whole experiment. PBL and CD4- or CD8-enriched subpopulations were cultured at 1 $\times$ 10⁶ cells ml⁻¹ in TCM in upright 25-cm² flasks (Corning T4160) for 4 d with or without VIT13 and 9.6. For activation the 9.6 mAb (provided by Paul Martin) was used at 1:5000 dilution of ascites fluid and VIT13 (provided by Walter Knapp) at 5 μ g ml⁻¹. After 4 days of incubation, the cells were counted and tested for ³H-thymidine incorporation and cytotoxicity as follows. Cells (1 $\times$ 10⁵) were placed in U-bottom microtitre plates (Costar), pulsed for 8 h with 0.2 μ Ci of ³H-thymidine (ICN Radiochemicals), then harvested onto glass fibre paper and counted in an LKB β -counter (Turku, Finland). Epstein-Barr-virus-transformed lymphoblastoid cell line (LCL), MJ4, was maintained in culture in RPMI 1640 with 10% fetal bovine serum. Cells were seeded at 0.5 $\times$ 10⁶ ml⁻¹ and aliquots were recultured in fresh medium twice a week and used as targets in LDCC. LDCC was performed by adding 0.05 ml of a 500 μ g ml⁻¹ stock solution of PHA-M (Difco) to 0.1 ml TCM in every experimental well, so that the final concentration of PHA was 1%. The effector/target cell ratios ranged from 30:1 to 1:1. The degree of lysis was calculated as described previously²¹. The remaining cells were extensively washed, stained with FITC-conjugated OKT4 or OKT8 and positively sorted by flow cytometry gating on CD4⁺ or CD8⁺ lymphoblasts; activated PBL were sorted according to their size. The positively-sorted blasts were then recultured with or without the following cytokines: rIL-2 (100 U ml⁻¹, Cetus Corporation) or rIFN- γ (100 U ml⁻¹, Genzyme). Cells at the end of culture were essentially 100% viable.

Fig. 2 *a*, Proliferation of CD8⁺ cells purified as in Fig. 1 legend. *b*, Tac (IL-2 receptor) expression studied by indirect immunofluorescence. CD8⁺ cells (1 $\times$ 10⁶) were activated with rIL2 ($\square$), OKT3 ($\circ$), VIT 13 and 9.6 (Δ), or TCM ($\blacktriangle$), incubated at 4°C for 30 min with 100 μ l of antibody against Tac (from Dr Thomas Waldmann) at 1:10,000 dilution. Cells were then washed three times with cold 5% HS-PBS and resuspended in 100 μ l PBS containing 50 μ g ml⁻¹ of propidium iodide (Calbiochem-Behring) to stain dead cells. Cells were analysed in a Becton and Dickinson FACS IV. *c*, CD8⁺ cells, treated with the stimuli described above, were tested after 4 days of culture in an LDCC assay (see Fig. 1 legend). Effector target ratios were as follows: solid bars, 30:1; single-hatched bars, 10:1; open bars, 3:1; cross-hatched bars, 1:1. *d*, CD8⁺ cells were cultured with or without stimuli as described above. Supernatant concentration: solid bars, 66%; single-hatched bars, 22%; open bars, 7%; cross-hatched bars, 2%. Supernatants were harvested after 24 h of activation and filtered (similar results were obtained up to 96 h of culture (data not shown)). CTLL-2, and IL-2-dependent T lymphocyte line, was washed free of T-cell growth factor (TCGF, Biotest, Frankfurt) in which it is maintained and plated at 1 $\times$ 10³ cells per well (0.15 ml total per well). Cultures were incubated at 37°C for 24 h and pulsed for 8 h with 0.2 μ Ci of ³H-thymidine. All assays were done in triplicate. TCGF was used as a positive control. Control indicates ³H-thymidine incorporation in the presence of fresh TCM.



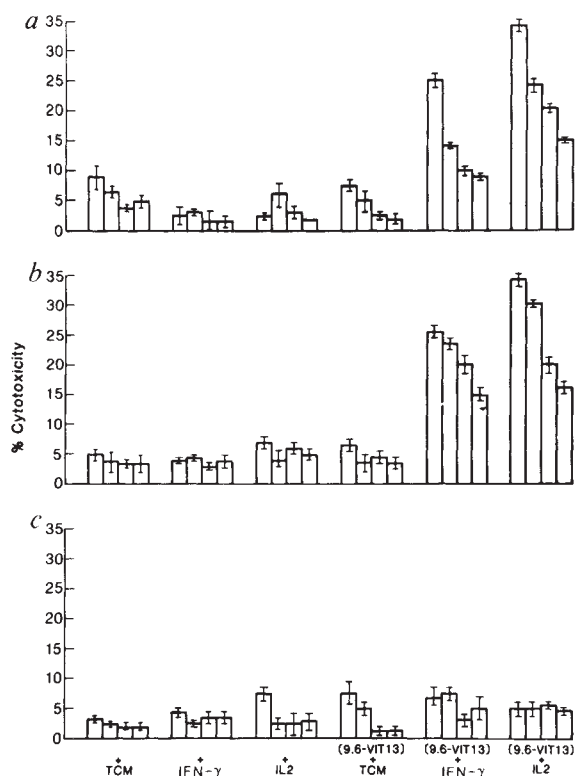


Fig. 3 PBL (top) CD8⁺ (middle), or CD4⁺ (below) blasts pre-treated with three groups or without (right hand three groups) VIT 13 and 9.6 were tested in LDCC after 1, 2 or 3 days of incubation with rIL-2, rIFN- γ or TCM. Lytic activity on day 2 is shown. Proliferative responses of the sorted cells with or without VIT13 and 9.6 incubated with rIFN- γ or TCM were always less than 1,000 c.p.m. Representative responses after the addition of rIL-2: 16,000 c.p.m. in the cells preactivated with VIT 13 and 9.6 and 3,000 c.p.m. in the controls. No differences in the proliferative response were observed among PBL, CD4⁺ or CD8⁺ positively sorted cells. Effector/target ratios in each group were (left to right) 30:1, 10:1, 3:1 and 1:1. Methods are as in Fig. 2 legend.

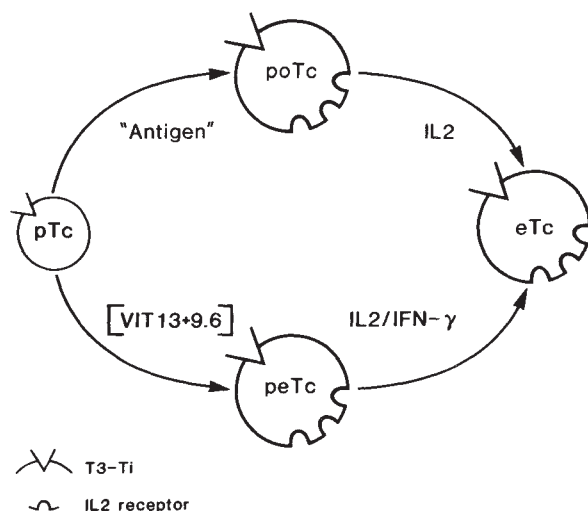


Fig. 4 Proposed model for functional maturation of T lymphocytes. See text for details.

we speculate that IL2R⁺ precursors would show the response noted. Also, although it is attractive to suggest that the poised T lymphocyte would precede the peTc intermediate in the maturation pathway, it is not established that this is so, nor even that the two cells are found in a single pathway. It must be remembered that cells at the peTc stage do not appear to produce IL-2 whereas we suggest, but have not shown, that the antigen-

stimulated cells would. Nakagawa *et al.* have recently proposed a very similar model for B-cell activation¹⁹. The techniques of molecular biology combined with further cellular studies should help to resolve some of these issues.

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HLA-DQ is epistatic to HLA-DR in controlling the immune response to schistosomal antigen in humans

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Antigens that produce an antibody response in some members of a species may fail to do so in others. The response to an antigen is controlled by a gene termed the immune response (*I*) gene, which is transmitted as a single dominant trait. We have provided evidence for similar immune suppression (*I*_s) genes which control non-responsiveness through the antigen specific suppressor T cell¹⁻⁶. The non-responsiveness is also dominantly inherited and the *I*_s genes are linked to the histocompatibility (HLA) antigen system. Here we report that the HLA-DR2 molecule from a non-responder haplotype (HLA-Dw12-DR2-DQw1) is required for the proliferative T cell response to schistosoma japonicum (Sj) antigen, as a restriction element, indicating that the HLA-DR2 is the product of the *I*_s gene, and that the HLA-DQw1 molecule of the non-responder haplotype is important in the antigen-specific suppression of the response to this antigen, suggesting that it is the product of the *I*_s gene. We therefore conclude that the HLA-DR and DQ molecules, which are controlled by the distinct genes in the MHC multigene family, regulate immune response and immune suppression and that the gene for HLA-DQ is epistatic to that for HLA-DR in controlling the immune response to schistosomal antigen in humans.

We reported previously a significant increase in the frequency of HLA-Dw19-DRw13-DQw1 haplotype and a significant

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Table 1 Sj antigen-specific T-cell lines from high responders and non-responders

T-cell line donor and HLA phenotype	Cells	Sj	Immune Response (c.p.m.) to PPD	SCW	medium
Y.Y. (non responder)					
DR2, -	WMN	1,827	157,865	1,827	2,711
Dw12, -	T-cell line	509	NT	NT	2,025
DQw1, w3	APC alone	336	NT	NT	467
	T-cell line + APC	54,746	4,077	1,352	1,269
H.A. (high responder)					
DRw13, -	WMN	51,166	80,163	4,012	3,921
Dw19, -	T-cell line	1,017	NT	NT	868
DQw1, w3	APC alone	463	NT	NT	526
	T-cell line + APC	21,681	2,598	1,110	3,129

Three individuals who had previously had infections of *Schistosoma japonicum* were investigated. All were born and raised in Yamanashi, Japan, where schistosomiasis japonica was endemic. They had been diagnosed as cases of schistosomiasis by stool examination, skin test and the circum-oval precipitin test. During the past decade, schistosomal eggs in the faeces had been absent. From epidemiological surveys, they are thought to have been exposed to *S. japonicum* for at least 10 years. Both non-responders (Y.Y. and S.H.) showed no complications of chronic schistosomiasis, but the high responder (H.A.) had post-schistosomal liver cirrhosis. HLA-A, B, C, typing was performed by the NIH standard microcytotoxicity method²³. HLA-D typing was by mixed lymphocyte culture reaction, and HLA-DR was typed as described elsewhere¹. Sj antigen was extracted from adult worms of *S. japonicum* by the method of Chaffee *et al.*²⁴ with minor modification. Controls were streptococcal cell-wall antigen (SCW) extracted as described elsewhere¹, and purified protein derivative (PPD) (Nihon BCG Seizo, Tokyo). For the proliferation assay, peripheral blood whole mononuclear cells were prepared by the Ficoll-Conray density-gradient method described elsewhere¹. 1×10^5 mononuclear cells were suspended in 0.2 ml culture medium (RPMI 1640 medium supplemented with 10% inactivated human male sera, blood type AB) and were cultured with or without antigen (Sj, $10 \mu\text{g ml}^{-1}$; PPD, $5 \mu\text{g ml}^{-1}$; SCW, $5 \mu\text{g ml}^{-1}$) for 7 days at 37°C in a mixture of 5% CO_2 and 95% air, in a humidified incubator. On day 6 of culture, $1 \mu\text{Ci}$ of ^3H -thymidine (NEN, 6.7 Ci mmol^{-1}) was added to the culture. The incorporation of ^3H -thymidine was measured using a scintillation counter. To generate the antigen-specific T-cell lines, T cells were purified by E-rosetting and were subdivided into CD4^+ and CD4^- T cells by panning²⁵. Dish-adherent cells were used as macrophages. Freshly separated CD4^+ T cells and macrophages were suspended in culture medium with $10 \mu\text{g ml}^{-1}$ schistosomal adult worm antigen (Sj) and plated in 6-well microtitre plates (Nunc), then cultured for 6 days at 37°C in 5% CO_2 and 95% humidified air. T-cell blasts were collected and suspended with autologous irradiated (3,000 R) mononuclear cells as feeder cells and with $10 \mu\text{g ml}^{-1}$ Sj in RPMI 1640 medium containing 10% HS and 10% MLA-144 culture supernatant as exogenous IL-2²⁶. These T-cell blasts were plated in 24-well microtitre plates (Linbro) and cultured at 37°C in a CO_2 incubator. Culture medium was changed every 2 days and feeder cells were added to the culture every 10 days. The established T-cell lines were cultured for at least 20 days before the proliferation assay. Cells of the T-cell line (1×10^4) and autologous or allogeneic irradiated (3,000 R) mononuclear cells as antigen-presenting cells (5×10^4) were suspended in 0.2 ml 20% human serum RPMI 1640 medium, plated in 96-well flat-bottomed plates (Falcon) and cultured for 72 h at 37°C in a humidified 5% CO_2 incubator. For the last 12 h, $1 \mu\text{Ci}$ of ^3H -thymidine was added and the incorporated ^3H -thymidine was counted, using a liquid scintillation counter. The median c.p.m. of the triplicate cultures was used for analysis. NT, not tested.

decrease in the frequency of HLA-Dw12-DR2-DQw1 haplotype in 121 patients with post-schistosomal liver cirrhosis (PSLC) and in 46 high responders to Sj antigen⁷. Patients with PSLC showed a high response to Sj antigen, in terms of the T-cell proliferative response, *in vitro*. The low response to Sj antigen, statistically associated with HLA-Dw12-DR2-DQw1, was mediated by CD8^+ suppressor T cells specific for Sj antigen^{3,5}. Thus, the HLA-Dw12-DR2-DQw1 haplotype is a non-responder haplotype to Sj antigen that controls resistance to PSLC, through antigen-specific suppressor T cells.

To investigate the function of HLA molecules in the regulation of the human immune response *in vitro*, we first established interleukin-2 (IL-2)-dependent, Sj antigen-specific, long-term cultured CD4^+ T-cell lines (helper-type T cells) from both non-responders and high responders to Sj antigen. The cell-surface markers of the T-cell lines were CD4^+ , 8^- , 25^+ (ref. 8), HLA-DR⁺ and HLA-DQ⁺. Both CD4^+ T-cell lines established from Y.Y. (non-responder) and H.A. (high responder) showed a good proliferative response to the Sj antigen, in the presence of autologous antigen-presenting cells (Table 1). No significant response occurred with other nominal antigens, such as purified protein derivative (PPD) or streptococcal cell wall (SCW) antigen, indicating that the CD4^+ T-cell lines were specific for Sj antigen. Only those antigen-presenting cells that express HLA-DRw13 co-operate with the T-cell line from the high responder H.A. (who was heterozygous for the high-responder HLA-Dw19-DRw13-DQw1 haplotype) to give increased proliferation. This response was markedly blocked by the anti HLA-DR framework monoclonal antibody (mAb)⁹, but was not affected by antibodies against HLA-DQw1 (ref. 10) or HLA-DP (ref. 11, Table 3). The T-cell line from non-responder Y.Y., who was heterozygous for the non-responder HLA-Dw12-DR2-DQw1 haplotype showed a significant response to Sj antigen only when the allogeneic

APC shared the HLA-DR2 antigen with the T-cell line donor (Table 2). Table 3 shows that the proliferation of T-cell lines from Y.Y. in response to antigen presented by either autologous or HLA-DR-matched allogeneic APC was blocked by the anti HLA-DR framework mAb but not by anti HLA-DQw1 or by mAbs against HLA class I¹². These findings confirm that the HLA-DR2 molecule acts as a restriction molecule in the presentation of antigen to CD4^+ T cells, even though this HLA-DR2 molecule is from non-responder haplotype. Taken together, we conclude that the DR molecules of both the high responder haplotype (Dw19-DRw13-DQw1) and the non-responder haplotype (Dw12-DR2-DQw1) are restriction molecules in the response to the Sj antigen. Our previous study clearly demonstrated that the low response to Sj antigen is mediated by CD8^+ suppressor T cells⁵, suggesting that the difference in the immune response to Sj antigen between the high-responder and low-responder haplotype is not related to the ability to present Sj antigen to T cells, but rather to the ability to induce an antigen-specific suppression.

To investigate the function of HLA molecules in immune suppression, we performed a blocking study, using various mAbs against HLA antigens. Whole mononuclear cells of non-responder S.H. or Y.Y. were cultured, with or without antigens, in the presence of various mAbs for 7 days. As shown in Table 4, such cells, even those derived from the non-responder S.H., showed a significant response when mAbs against DQw1 (HU-11¹⁰, SDR4.1¹³, or SDR1.2¹⁴) were added at the start of culture. The restored response in the non-responder was at the same level as that obtained when CD8^+ suppressor T cells were removed from the culture (for S.H., values were 4,278 c.p.m. without suppressor T cells and 1,749 c.p.m. with; for Y.Y., 9,338 c.p.m. without suppressor T cells, 4,981 c.p.m. with). Restoration of the immune response was specific for the Sj

Table 2 HLA-DR restriction in the co-operation between Sj antigen-specific T-cell lines and allogeneic antigen-presenting cells (APC)

T-cell line donor and HLA phenotype	Combination	APC donor	HLA-DR	Immune response (c.p.m.) to		
				Sj	medium	Δ c.p.m.
Y.Y. (non-responder) Dw12, - DR2, - DQw1, 3	Autologous A	KF	2, w9	12,317	1,515	10,802
		60114	2, 5	26,090	336	25,754
		SM	2, w6	16,222	691	15,531
		6034	1, 2	12,644	1,288	11,356
		KT	1, 2	12,462	1,150	11,312
		6031	2, 5	12,057	1,152	10,905
		5801	2, w9	11,355	474	10,881
		TOK	1, 2	10,169	334	9,835
		TN	2, 2	9,091	1,269	7,822
		MH	2, w8	7,592	450	7,142
	B	YAS	2, 4	7,861	1,622	6,239
		MS	4, 4	2,291	695	1,596
		RO	1, w13	3,367	1,957	1,410
		Shim	w13, w8	1,877	948	929
		IK	w8, w8	2,551	1,818	733
		5954	4, w8	1,785	1,244	541
H.A. (high responder) Dw19, - DRw13, - DQw1, w3	A	MK	w8, w9	1,503	1,025	478
		6092	w9, w9	1,761	1,886	-125
			w8, w9	768	1,284	-516
	A	KY	2, w13	25,955	2,393	23,562
		6011	w13, w8	16,985	541	16,444
		SH	2, w13	16,409	1,844	14,565
		MS	1, w13	10,092	1,264	8,828
	B	TOK	2, 2	3,099	1,609	1,490
		FH	w8, -	3,132	1,653	1,479
		HS	1, 2	2,242	939	1,303
		CAH	1, 1	609	511	98

Combination A, allogeneic APC matched for HLA-DR; combination B: allogeneic APC that shares no HLA-DR with the T-cell line donor. Antigen-presenting cells are from healthy donors, apparently never exposed to *Schistosoma japonicum*. CAH is the homozygous typing cell for HLA-Dw1 of caucasoid origin and was donated by Dr N. Reinsmoen, University of Minnesota. The proliferation assay for T-cell lines is described in the legend of Table 1.

antigen because the response to the SCW antigen was not affected by these mAbs. A similar antigen-specific restoration of the immune response was observed when whole mononuclear cells from non-responder Y.Y. were challenged with Sj antigen in the presence of HU-11 (matched for DQw1). Because the mAb against DQ did not directly enhance the response of the T-cell line to Sj antigen (Table 3) or of mononuclear cells to other nominal antigens, it is most probable that the mAb against DQw1 blocked the Sj antigen-specific immune suppression. This observation raises the possibility that the HLA-DQw1 molecule in the non-responder haplotype HLA-Dw12-DR2-DQw1 is the product of the *Is* gene specific for Sj antigen. The DQ molecule is more polymorphic than the DR molecule, as shown by two-dimensional gel electrophoresis¹⁵ or by cloning *HLA* genes¹⁶. In fact, the HLA-DQw1 molecules in the HLA-Dw12-DR2-DQw1 and Dw19-DRw13-DQw1 haplotypes (unpublished observation) are different although both are serologically typed as DQw1. This suggests that the difference in structure of the DQw1 molecule is responsible for the difference in immune response to Sj antigen between non-responder and the high-responder haplotypes. Thus, in the non-responder haplotype, the DR2 molecule can present antigen to CD4⁺ T cells, increasing the response to the Sj antigen, but this response is suppressed by suppressor T cells controlled by the DQw1 molecule in the non-responder haplotype. In other words, HLA-DQ is epistatic to HLA-DR in regulation of the immune response to Sj antigen. In laboratory animals, the immune response under *Ir*- and *Is*-gene control has been extensively investigated¹⁷⁻¹⁹. The class II molecules encoded by the genes in the MHC in mice (where

the I-A molecule is comparable to the DQ molecule and the I-E molecule comparable to the DR molecule) act as restriction elements in the activation of helper T cells and suppressor T cells, respectively, when lactate dehydrogenase B(LDH_B)²⁰, IgG_{2a} myeloma protein²¹ or F antigen²² is used as the challenging antigen. In these systems, antigen is presented to helper T cells by the I-A molecule, whereas suppressor T cells are stimulated by antigen in the context of the I-E molecule. Although gene organization in the HLA-D region in humans is much more complicated than that of the murine H-2 I region, it seems that both in mice and humans, one of the two molecules encoded by tandemly duplicated class II loci controls the immune response, and the other controls the immune suppression. Duplicated class II loci presumably diverged in function, corresponding to the appearance of the functionally distinct T cells, helper T cells and suppressor T cells in the immune system. The immune response to many pathogens is essential for the survival of humans, yet the immune response is not without adverse effects. An analysis of the immune response of the human population sensitized to exogenous complex antigens by natural exposure is not ideal, but our findings that HLA-DR and DQ molecules have distinct functions in the immune regulation provide a clue to the need for generation of a multigene family of MHC and to the mechanisms of immune regulation and disease susceptibility.

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Table 3 Effects of anti-DR and anti-DQ mAb on the response of T-cell lines to Sj antigen

T-cell line donor and HLA phenotype	mAbs	Sj	Immune response (c.p.m.) to medium		Δ c.p.m.
Y.Y. (non-responder)					
DR2, -	Expt. 1	(-)	9,488	150	9,338
Dw12, -		HU-4(DR)	1,567	216	1,351
DQw1, w3		HU-11(DQw1 + α)	9,641	1,931	7,710
		w6/32 (A, B, C)	7,204	568	6,636
	Expt. 2	(-)	9,247	1,028	8,219
		HU-4(DR)	3,175	1,575	1,600
		HU-11(DQw1 + α)	7,398	1,336	6,062
		HU-18(DQw3)	6,938	1,567	5,371
H.A. (high-responder)					
DRw13, -		(-)	12,515	1,245	11,270
Dw19, -		HU-4(DR)	2,859	539	2,320
DQw1, w3		HU-11(DQw1 + α)	10,006	1,354	8,652
		HU-18(DQw3)	13,542	2,256	11,286
		9C4(DP)	9,283	874	8,409

The T-cell line from non-responder Y.Y. was co-cultured with autologous irradiated peripheral blood lymphocytes (PBL) and Sj antigen. The proliferation assay was performed as described in the Table 1 legend. Murine mAb HU-4, HU-11, and HU-18 (ref. 27) were donated by Dr M. Aizawa, Sapporo. The mAb HU-4, specific for HLA-DR framework was described by Koide *et al.*⁹. The mAb 9C4 (anti-DP framework monoclonal antibody) was kindly donated by Dr M. Katagiri, Asahikawa. Anti-class I mAb w6/32 was purchased from Sera-Lab (United Kingdom). All mAbs used here were prepared from ascites and their reactivity was confirmed by adequate indirect immunofluorescence staining of B-lymphoblastoid cell lines. HU-4, the mAb against HLA-DR was proved to have no toxic effect by chromium release assay (data not shown). The other mAbs were not checked for their cytotoxicity, but the cytotoxic effect of those mAbs should be negligible when we use them in the culture because they did not inhibit the proliferative response to nominal antigens and lectins, such as purified protein derivative (PPD), *Candida albicans* antigen or phytohaemagglutinin (PHA). The mAbs HU-4(DR), HU-11(DQw1) and HU-18(DQw3) are available on request from Dr M. Aizawa, Department of Pathology, School of Medicine, Hokkaido University, Sapporo, Japan.

Table 4 Restoration of the lymphoproliferative immune response of low responders by monoclonal antibodies against HLA-DQ

Exp. no.	PBL donor and HLA phenotype	Added antibodies (specificity)	Immune response (c.p.m.) to			Δ (c.p.m.)	
			Sj	SCW	Medium	Sj	SCW
1	S.H. (non-responder)	—	2,915	NT	2,397	518	NT
	DR2, w13	—	16,066	NT	1,470	14,596	NT
	Dw12, w19	HU-11 (DQw1 + α)	25,453	NT	2,117	23,336	NT
	DQw1, -	SDR4.1 (DQw1)	2,806	NT	1,844	962	NT
		HU-4 (DR monomorphic)	2,331	NT	2,086	245	NT
		W6/32 (class I)	4,371	20,213	3,070	1,301	17,143
2	S.H.	—	12,953	27,298	4,214	8,739	23,084
		HU-11 (DQw1 + α)	7,003	8,286	1,672	5,331	6,614
		SDR4.1 (DQw1)	9,992	12,461	3,507	6,485	8,954
		SDR1.2 (DQw1)	3,411	30,006	3,388	23	26,618
		HU-18 (DQw3)	3,017	21,008	932	2,085	20,076
		9C4 (DP mono)	2,138	1,148	670	1,468	478
		HU-4 (DR mono)					
3	Y.Y. (non-responder)	—	2,638	NT	2,711	-73	NT
	DR2, -	—	31,516	NT	2,060	29,456	NT
	Dw12, -	HU-11 (DQw1 + α)	1,436	NT	933	503	NT
	DQw1, w3	W6/32 (class I)					
4	Y.Y.	—	854	990	890	-36	100
		HU-11 (DQw1 + α)	9,071	1,851	1,215	7,856	636
		HU-18 (DQw3)	1,756	2,390	2,303	-547	87
		9C4(DP)	2,194	1,781	1,361	833	420
		W6/32 (class I)	1,230	1,507	1,322	-92	185

The mAbs SDR4.1 and SDR1.2 specific for HLA-DQw1 were gifts from Dr J. G. Bodmer, London. The origin of the other mAbs used here is as in Table 3 legend. In the co-culture experiments, mAb was added at the start of culture at the final dilution of 1:300. The proliferation assay is described in the Table 1 legend. HU-18 was not reactive to B cells of SH. NT, not tested.

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A point mutation at codon 13 of the N-ras oncogene in myelodysplastic syndrome

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Patients with a myelodysplastic syndrome (MDS) which has a risk of leukaemic change exhibit a variable clinical course¹⁻³. It has been suggested that the development of leukaemia in patients with MDS may be related to chromosomal abnormalities or genetic alterations³⁻⁵: somatic mutation of the N-ras gene is now considered to be a critical step in the genetic basis of human leukaemogenesis⁶⁻¹⁴. Here we report that DNAs of bone-marrow cells from three out of eight patients with MDS contained an activated N-ras oncogene, as detected by an *in vivo* selection assay in nude mice with transfected NIH 3T3 cells^{12,15}. Molecular analysis revealed the same single nucleotide substitution at codon 13 in all three transforming N-ras genes. Each of the three patients showed a progression of the disease and a resulting leukaemic change within the following year. Our observation of the mutation at codon 13 in leukaemic cell DNAs from all three cases suggests that activation of the N-ras gene is important in the development of leukaemia in some MDS cases.

Eight patients with MDS, including four patients with refractory anaemia (RA) and four patients with RA with excess of blasts (RAEB), were investigated. The diagnosis of MDS fulfilled the criteria of the FAB classification¹⁶. Table 1 shows the clinical characteristics of the patients at the time of transfection analysis. These data are essentially the same as those obtained at the first diagnosis. DNAs from bone-marrow cells were tested for the presence of transforming genes by a direct *in vivo* selection assay¹², a modification of the tumorigenicity assay of Blair *et al.*¹⁵. DNA samples were used to co-transfect NIH 3T3 cells with the plasmid pSV2Neo (ref. 17), and transfected cells selected with the antibiotic G418 were injected into subcutaneous sites of nude mice. Tumours arose 40-70 days after inoculations of NIH 3T3 cells transfected with DNAs from three out of eight MDS patients. Eight normal human DNAs

tested in the same way failed to give any tumours within 80 days (Table 2).

All tumours that arose in nude mice were shown to contain a large number of human repetitive sequences by Southern analysis with the probe Blur-8 (ref. 18) (Fig. 1a). To examine whether any of these tumours resulted from the acquisition of a human ras oncogene, DNA from each of the tumours was analysed for N-ras, H-ras and K-ras sequences. All tumour DNAs derived from the three MDS patients were found to contain the human N-ras gene (Fig. 1b and Table 2).

For characterization of the N-ras genes detected, we cloned three genomic N-ras genes from the tumours derived from the three cases. The physical restriction map of each N-ras gene cloned was identical with that of the normal human N-ras gene^{19,20}. The enzyme *Eco*RI splits the N-ras gene into the 5' region of 9.2 kilobases (kb) and the 3' region of 7.0 kb (refs 19 and 20). To localize the site of the N-ras activation, chimaeric molecules between the normal and the transforming N-ras genes were constructed and tested for transforming activity. In all three cases, the chimaeric genes containing the 9.2-kb fragment of the transforming N-ras gene were active, whereas those containing the normal 9.2-kb fragment did not show detectable transforming activity, suggesting that the lesions responsible for activation of these N-ras genes are in their 9.2-kb fragments (Fig. 1c). The 9.2-kb fragment of an N-ras gene contains the first and second exons of the p21 coding sequence^{19,20}. Therefore, we determined the nucleotide sequence of the first and second exons of the transforming N-ras genes. Comparisons of their sequences with those of the normal N-ras gene revealed that the three transforming N-ras genes were activated by the same single point mutation from G to C at codon 13 of the predicted protein (Fig. 1d).

To compare the prognostic progression of the disease in patients with and without an activated N-ras gene in marrow cells, we observed clinical courses in all the patients during the following year. The development of acute myeloid leukaemia (AML) was observed in the three patients with the activated N-ras oncogene 6-12 months after the transfection assay, whereas significant progression of the disease was not observed in the other five cases (Table 2). To analyse whether the leukaemias which developed from the MDS samples also bear the same mutation at codon 13, synthetic 20-mer oligonucleotide probes were used to detect the mutation. The oligomer probe N13-1C, corresponding to the sequence of the N-ras gene mutated at codon 13, formed a fully matched hybrid with both the leukaemia cell DNAs from the three cases and the transformant DNAs derived from the MDS DNAs (Fig. 2). However, the N13-1C probe failed to give a clear hybridizing signal in the MDS DNAs because there were so few blasts in the MDS sample (data not shown). These observations indicate that the mutated N-ras genes have arisen by somatic mutation and that the development of leukaemia from MDS in three cases is related

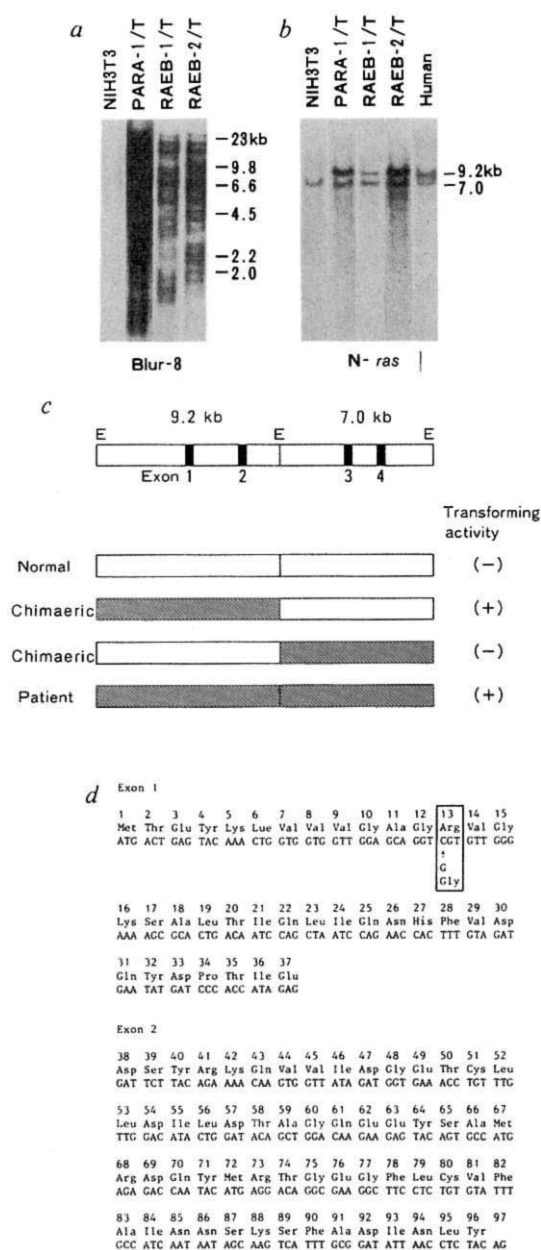


Fig. 1 Identification and analysis of the *N-ras* oncogenes in the three MDS patients. **a**, **b**, Southern blot analysis to show the presence of human repetitive and *N-ras* sequences in NIH 3T3 transformants from MDS DNAs. Transformant DNAs (10 µg) were digested with *EcoRI*, electrophoresed, blotted and hybridized **a**, to the Blur-8 probe¹⁸ and **b**, to the *N-ras* probe²⁰ for 9.2-kb and 7.0-kb *EcoRI* fragments. Hybridization was as described previously¹³. **c**, Transforming activity of chimaeric *N-ras* genes in the three cases. Chimaeric gene constructions of the 9.2-kb and 7.0-kb *EcoRI* fragments were performed using T4 DNA ligase and gel-purified *EcoRI* fragments from the normal *N-ras* gene and the transforming *N-ras* genes. To assess the biological activity of the genes, the resulting concatamers were tested by an *in vivo* selection assay in nude mice using transfected NIH 3T3 cells. **d**, Nucleotide sequence and predicted amino-acid sequence of the entire first and second exons of the cloned *N-ras* genes in the three MDS patients. The complete nucleotide sequence of both the exons was identical in the three *N-ras* genes from the three MDS cases. A single base change (G to C) and the consequent amino-acid change of glycine to arginine are boxed. The known RNA splice sites are marked by arrows¹⁹. Sequences were determined by the chain termination method²³ after subcloning suitable restriction-enzyme-generated DNA fragments into M13 phage replicative form DNAs as described by Hirai *et al.*¹³.

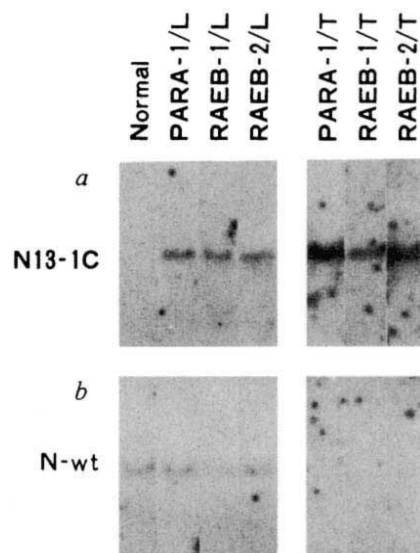


Fig. 2 Hybridization of synthetic oligomer probes to leukaemic cell DNAs (L) from the three patients in the leukaemia phase and to NIH 3T3 transformant DNAs (T) derived from DNAs in the MDS phase. Normal human DNA was used as control. The panels were hybridized to oligomer probes: **a**, N13-1C (5'-GGAG-CAGGTCGTGTTGGGAA-3'); **b**, N-wt (5'-GGAGCAGGTG-GTGTGGGAA-3').

Methods. Synthesis of the probes and hybridization experiments were carried out as described by Bos *et al.*^{9,12}. The N13-1C and N-wt probes were synthesized using as template chemically synthesized 20-mer and an 8-mer primer complementary to the 3' end of the 20-mer. This primer-template mixture was incubated with [α -³²P]dGTP (3,000 Ci mmol⁻¹), [α -³²P]dTTP (3,000 Ci mmol⁻¹), cold dCTP and dATP, and DNA polymerase I. Because of the 5' phosphate groups on the 8-mer primer, the labelled oligomer was separated from the unphosphorylated template on a 10% polyacrylamide-7M urea gel. The labelled oligomer was visualized by autoradiography, the excised band was eluted in 1 mM EDTA and the eluate was used for hybridization. Genomic DNA (10 µg) was digested with *PstI* and electrophoresed on a 0.5% agarose gel. The gels were denatured in 0.4 M NaOH, 0.8 M NaCl, neutralized in 0.5 M Tris-HCl (pH 7.4), 1.5 M NaCl and dried. The dried gel membranes were hybridized at 53 °C with each probe in 5 × SSPE (1 × SSPE is 10 mM sodium phosphate pH 7.0, 0.18 M NaCl, 1 mM EDTA), 0.3% SDS and 10 µg ml⁻¹ sonicated salmon sperm DNA. Hybridized gels were washed in 2 × SSPE, 0.1% SDS at room temperature, in 5 × SSPE, 0.1% SDS at 53 °C for 15 min and finally in the same solution at 63 °C for 5 min. Membranes were autoradiographed for 3–5 days using intensifying screens.

to an increase in cells containing the mutated *N-ras* gene. The oligomer N-wt corresponding to the normal sequence of the *N-ras* gene did not form a fully matched hybrid with any of the transformant DNAs, although it hybridized to the leukaemia cell DNAs. The high proportion of blast cells in the leukaemia samples makes it likely that the band hybridizing with the N-wt probe is due to the leukaemic cells containing both a normal and a mutant *N-ras* allele.

In most of the cases examined, activation of a human *N-ras* gene depends on a point mutation in either codon 12 or 61 of the gene^{9,11,13,19–22}. Similarly, mutations at codon 12 or 61 have been detected in some AML cases and cell lines^{9,11,12} and recently in our five AML samples tested by *in vivo* selection assays (not shown). As exceptions, Bos *et al.* have observed a mutation at codon 13 of the *N-ras* gene in four patients with AML by *in vivo* selection assays¹², but it is not known whether these AML cases had developed from MDS cases. The *N-ras* gene in the four AML cases had a GAT or GTT triplet at codon 13, whereas the three *N-ras* genes described here had a CGT codon 13. A standard *in vitro* focus formation assay revealed

Table 1 Clinical characteristics of patients at the time of transfection assay

Case	Age, sex	Disease duration (months)	Peripheral blood			Bone marrow		
			Hb (g dl ⁻¹)	WBC (μl ⁻¹)	Plt (×10 ⁴ μl ⁻¹)	Cellularity	Blast (%)	Cytogenetics
PARA-1	34 M	30	6.0	2200	10.9	H	<5	46XY, 5q-
PARA-2	72 F	33	8.1	3400	5.1	H	<5	46XX
PARA-3	23 F	8	7.9	3000	8.8	N	<5	47XX, +8
PARA-4	29 M	49	7.4	3300	2.8	H	<5	46XY
RAEB-1	40 M	40	11.7	2800	8.1	H	5.7	46XY
RAEB-2	54 M	11	8.2	2600	7.4	N	9.0	44XY, -5, -17
RAEB-3	57 F	9	5.0	1100	0.7	H	6.8	45XX, -7
RAEB-4	50 M	29	6.2	1900	7.2	N	5.2	45X, -Y

Hb, haemoglobin; WBC, white blood cells; Plt, platelets; H, hypercellular; N, normocellular.

Table 2 Transforming gene and clinical progression in MDS cases

Case no.	Tumour incidence per co-transfection of 20 μg DNA	Transforming genes	Diagnosis after one year
PARA-1	0/4, 1/4	N-ras	AML
PARA-2	0/4, 0/4	—	NC
PARA-3	0/4, 0/4	—	NC
PARA-4	0/4, 0/4	—	NC
RAEB-1	1/4, 1/4	N-ras	AML
RAEB-2	1/4, 1/4	N-ras	AML
RAEB-3	0/4, 0/4	—	NC
RAEB-4	0/4, 0/4	—	NC
Normal human	0/16, 0/16	—	—

NC, not changed.

High-molecular-weight DNAs were extracted from buffy coat cells of heparinized marrow from the patients and the normal subjects. The transforming activity of the DNAs was assayed by transfection of NIH 3T3 cells using an *in vivo* selection method^{12,15}. Briefly, 20 μg cellular DNA and 300 ng pSV2Neo were precipitated with calcium phosphate into each of five 60-mm plates seeded one day previously with 2 × 10⁵ NIH 3T3 cells. After 20–24 h, the co-precipitate was washed off and the cells were incubated in growth medium (Dulbecco's modified Eagle's medium supplemented with 10% calf serum) for 20–24 h. Each dish was trypsinized and cells were divided into three dishes containing selective medium with 400 μg ml⁻¹ G418. After 7–10 days of growth in selective medium, 1.5 × 10⁶ cells were then injected into each of two inguinal subcutaneous sites of *nu/nu* mice.

that NIH 3T3 cells transfected with the cloned N-ras gene containing a CGT codon 13 did not display a striking change in morphology and that they showed relatively slow proliferation (data not shown). Although it is unclear whether these results are associated with the slow progression of the clinical course in MDS, our observations suggest that activation at codon 13 of the N-ras gene can be detected in some of MDS patients and that this activation may be related to leukaemic conversion in some MDS cases.

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A possible function for thaumatin and a TMV-induced protein suggested by homology to a maize inhibitor

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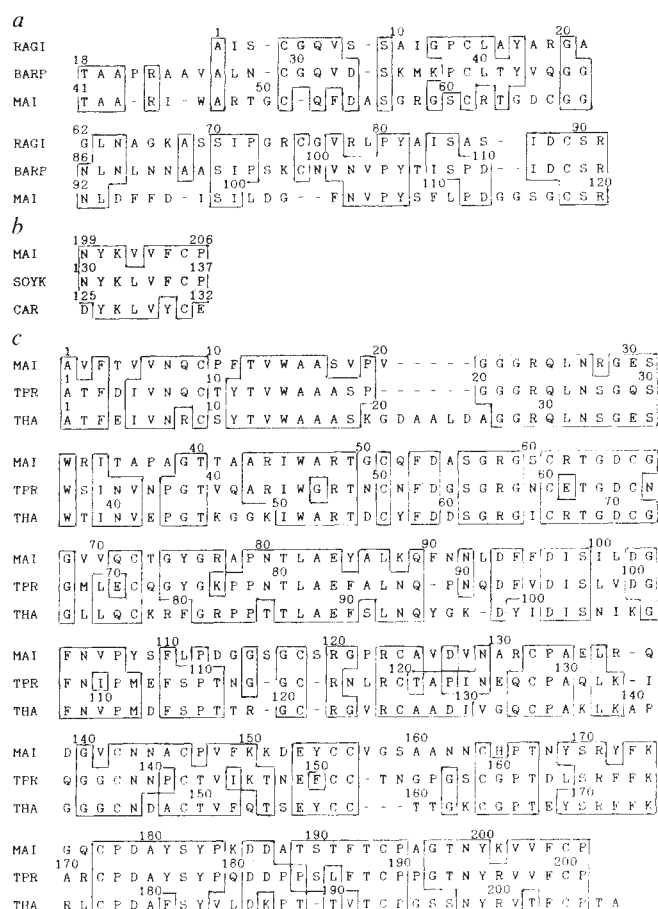
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Protein inhibitors of enzymes, which have potentially deleterious effects in animal nutrition and possible defensive functions against insect and microbial pests, are of widespread occurrence in plants^{1,2}. Because cereals provide about 55% of direct human protein intake their inhibitors have received much attention³. The amino-acid sequences of α-amylase inhibitors in wheat^{4–6}, and ragi (*Eleusine coracana*)⁷; proteinase inhibitors from maize⁸ and barley^{9,10}; and bifunctional proteinase/amylase inhibitors in ragi¹¹, barley^{12,13} and wheat¹⁴ show varied evolutionary relationships. Here we describe the sequence of a maize protein which is a potent *in vitro* inhibitor of bovine trypsin and the α-amylase from *Tribolium castaneum* beetles. The protein has little similarity to any of the other enzyme inhibitors, but is highly similar to the intensely sweet protein thaumatin¹⁵ and to a protein induced in tobacco following infection by tobacco mosaic virus (TMV)¹⁶. This unexpected finding suggests a possible function for these proteins.

The maize bifunctional inhibitor contains 206 amino acids corresponding to a relative molecular mass (*M_r*) of 22,077, and is notable for its high content of 16 cysteine residues. When the

Fig. 1 Alignments of the amino-acid sequence of the maize 22K bifunctional trypsin/ α -amylase inhibitor (MAI), *a*, with the sequences of a barley proteinase inhibitor (BARP)¹² and the ragi α -amylase inhibitor (RAGI)⁷; *b*, with the Kunitz-type proteinase inhibitors from soybean (SOYK)¹⁸ and *Adenanthera pavonina* (CAR)¹⁹; *c*, with a pathogenesis-related protein induced in tobacco by infection with TMV (TPR)¹⁶, and the sweet-tasting protein thaumatin (THA)¹⁵. Homologous amino acids are enclosed in boxes. Gaps (—) are introduced to facilitate the alignments.

Methods. The inhibitors in seeds of maize (line B8) were extracted from defatted flour with 0.1 M NaCl and partially purified by precipitation with ammonium sulphate (0–60%) and molecular sieve chromatography on columns (1.6×75 cm) of Sephadex G-75 as described previously³⁰. Final purification of the 22K bifunctional inhibitor of bovine trypsin and *Tribolium* α -amylase was achieved by reverse phase HPLC on a Vydac column (4.6 mm×25 cm). Samples (5 mg) were injected onto the column in 6 M guanidine-HCl in 0.1% trifluoroacetic acid (TFA) and eluted with a linear gradient of acetonitrile in 0.1% TFA. The 22K inhibitor eluted at a concentration of 39% acetonitrile. It had no inhibitory activity towards the α -amylases from mammals and bacteria. The amino-acid sequence of the protein was determined by the DABITC method³¹ applied to the intact reduced and S-alkylated protein and peptides derived from it by separate enzymatic digestions with trypsin, chymotrypsin and the endoproteinase from *Staphylococcus aureus* V8. The mixtures of peptides produced by these methods were resolved by reverse phase HPLC as described above and in ref. 19. Amino-acid analyses were obtained using a Waters PICO-TAG system. Full details of the purification, characterization and sequence determination will be given elsewhere (S.V.R., M.R. and A.B.L., in preparation).



sequence was compared with those of other proteins stored in a databank (US National Biomedical Research Foundation 1986) by computer analysis using the FASTP and RDF programs¹⁷, there was limited identity of short regions (8–30 residues) with the inhibitors from ragi⁷ and barley¹² (Fig. 1*a*) and the Kunitz-type inhibitors found in soybean¹⁸ and *Adenanthera pavonina*¹⁹ (Fig. 1*b*) but the RDF program indicated that these partial homologies had little biological significance (values of $z < 4$)^{17,20}. However a striking and extensive similarity (52%, $z = 41.74$) was discovered when the maize inhibitor was aligned with thaumatin II, the intensely sweet protein from the fruits of *Thaumatococcus daniellii* Benth.¹⁵ (Fig. 1*c*). Moreover most of the alterations are conservative replacements. A similar level of sequence homology (57%, $z = 97.79$) was discovered between the maize inhibitor and a so-called PR protein induced in tobacco plants following infection with TMV¹⁶.

The similarity between thaumatin and the PR protein has been noted before¹⁶, and PR proteins are sometimes referred to as thaumatin-like (TL) to highlight the structural similarity between these proteins of uncertain function. The strong sequence similarity with the maize bifunctional inhibitor indicated here suggests that both of these other groups of proteins should be examined for their possible effects on a range of hydrolytic enzymes. Interestingly, other workers who have noticed a very weak resemblance between short sections of the sequences of the PR proteins and the Bowman-Birk type proteinase inhibitors from legume seeds were unable to detect any inhibition of trypsin by the PR proteins²¹.

Mechanical wound-damage²², microbial infection²³ and insect attack²⁴ lead to greatly enhanced levels of certain proteinase inhibitors in plants. This phenomenon has been described as a 'primitive immune response' contributing to the overall defence of the plants, by reducing rates of endogenous mobilization of stored reserves, or by directly inhibiting the enzyme systems of

the invading pathogen or pest²⁵. Similarly the appearance of the pathogenesis-related (PR) proteins in response to viral infection has been associated with the development of 'systemic acquired resistance'²⁶. The present findings indicate that some PR proteins may not be directly anti-viral, but perhaps merely part of a general defence reaction.

The excessive sweetness of the thaumatins is associated with their high content of lysine residues and the specific amino acids interacting with taste receptors are Tyr 95, Lys 97 and Lys 106 (refs 27–29). It is perhaps noteworthy that despite its strong homology with the thaumatins, the maize M_r 22,000 (22K) inhibitor has no such obvious sweet taste, and contains only 6 Lys residues in its sequence instead of the 11 found in thaumatin¹⁵. Also the Tyr and Lys residues in the important positions 95, 97 and 106 are replaced by Phe, Asn and Asp respectively. We venture to suggest that the maize 22K bifunctional inhibitor is a potentially interesting target for protein and or genetic engineering studies, and have recently begun work in this direction in our laboratories.

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Novel submicroscopic extrachromosomal elements containing amplified genes in human cells

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In previous work¹, several methotrexate (MTX)-resistant variants were isolated from the human cell line HeLa BU25, which exhibited a high degree of dihydrofolate (DHFR) gene amplification (estimated to be 250- to 300-fold)^{1,2}. These variants did not contain any chromosome with a homogeneously staining region (HSR) and exhibited only a small average number of minute chromosomes per cell: these two types of karyotypic abnormalities generally accompany selective gene amplification³. We now report that structures containing amplified DHFR genes in one of these variants (HeLa BU25-10B3) can be isolated by pulsed-field gradient or field-inversion gel electrophoresis as homogeneous DNA molecules of ~650 kilobases (kb). Electron microscopy of metaphase spreads from these cells reveals chromatin fibres with a similar DNA content, which are probably related to the above elements. These represent a novel type of extrachromosomal structures in mammalian cells.

An experiment in which a metaphase chromosome preparation from 10B3 cells was fractionated in a sucrose gradient, and DNA from the individual fractions was tested with chromosome-specific probes showed that most of the DHFR-specific sequences are in structures sedimenting slower than chromosome 5, which contains the unamplified DHFR gene⁴, and also slower than chromosomes 13, 14, 15, 21 and 22, which contain the nuclear ribosomal RNA genes⁵ (data not shown). This experiment indicated that the amplified DHFR genes in these cells are in extrachromosomal structures not visible in the light microscope due to their small size.

Electrophoretic techniques using orthogonal^{6,7} or inverted-field pulses⁸ permit the separation of large DNA in the range 50-2,000 kb, such as the intact chromosomal DNAs from yeast^{6,7,9} and protozoa¹⁰⁻¹², and offered the possibility of isolating the putative submicroscopic extrachromosomal elements.

Samples of genomic DNA from HeLa S3 and HeLa BU25-10B3¹, and, for comparative purposes, from the MTX-resistant human cell variants VA₂B-6A₂-H¹, VA₂B-6A₃-B and VA₂B-6A₃-E, which contain abundant minute chromosomes (see legend of Fig. 1 and Table 1), were run in the pulsed-field gradient electrophoresis (PFGE) system (Fig. 1a,b). The ethidium bromide (EtBr) staining pattern (Fig. 1a) reveals, in the yeast DNA lanes, bands corresponding to individual chromosomes or unresolved chromosome doublets⁷⁻⁹. In the 10B3 DNA lane, a discrete band (thick arrow), absent in the other lanes, has migrated to a position between those of chromosomes V and VIII (~580 kb) and chromosome XI (~700 kb)⁹, and thus has a mobility corresponding to that of linear DNA of ~650 kb. The 10B3 DNA lane also contains fairly abundant DNA of heterogeneous size, of <300 kb, which possibly derives, in part at least, from degradation of larger molecules. The DNA samples from 6A₂, 6A₃-B and 6A₃-E cells show a band migrating approximately as yeast chromosome X (thin arrow), which is absent from the HeLa and 10B3 DNA samples, and which may be specific for the cell line VA₂-B and its derivatives.

The '650-kb' band of the 10B3 genomic DNA sample hybridized strongly with the human DHFR complementary DNA probe (pHD84; ref. 2), whereas the <300 kb heterogeneous DNA gave a much weaker signal with this probe (Fig. 1b). The 650-kb band most probably represents the extrachromosomal structures containing amplified DHFR genes revealed by the sucrose gradient fractionation experiment. Note that the minute chromosome DNA from this cell line and from the 6A₃-B, 6A₃-E and 6A₂ variants has not migrated from the origin.

As shown in Fig. 1c, changing the PFGE pulse length from 90 s to 60 s considerably affected the migration of the yeast chromosomal DNAs. The migration of the 10B3 650-kb elements was similarly affected. As it is known that small closed-circular DNAs, such as the supercoiled 2μ circles of yeast (6.3 kb) and supercoiled 45-kb cosmid DNAs, unlike linear DNAs, exhibit a mobility in PFGE which is independent of the pulse times (refs. 6, 12; E. Lai, unpublished data), the above observations strongly argued against the 650-kb elements being small supercoiled circles (see also below).

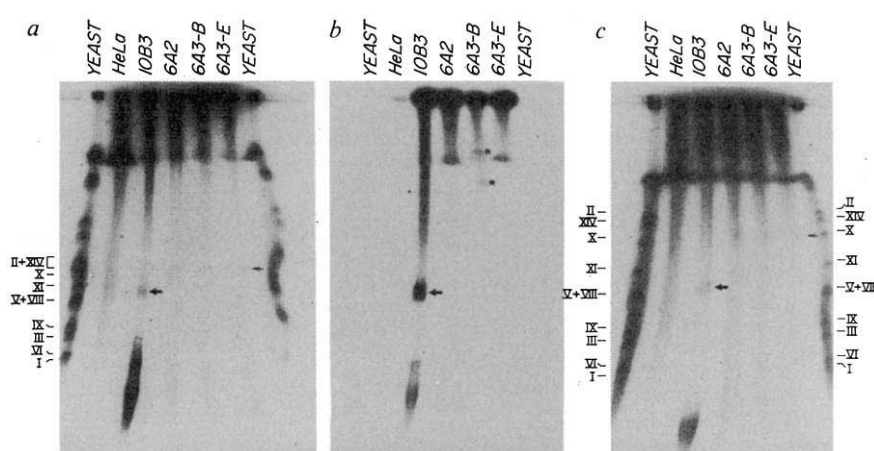
For an independent estimate of the size of the 650-kb elements, they were digested with the restriction enzyme *Sfi*I, and probed with nick-translated 650 kb DNA (Fig. 2). Both the EtBr staining pattern (Fig. 2a) and the autoradiogram (Fig. 2b) show three bands corresponding to components of ~370 kb, ~125 kb and ~75 kb in size, with no smaller fragment appearing in a shorter run (Fig. 2c). Assuming that each band contains one fragment, one can estimate for the extrachromosomal elements a size of ~570 kb. Digestion with *Xho*I produced six to eight bands in the range from ~30 to ~150 kb, which add up to the same approximate size as given above (not shown). These experiments, therefore, gave results consistent with the 650-kb elements having a size between 600 and 700 kb and a homogeneous structure.

When metaphase spreads from 10B3 cells, prepared as previously described¹³, were examined in the electron microscope many extrachromosomal structures consisting of 20- to 30-nm chromatin fibres arranged in most cases in a circular or a convoluted, more complex configuration, with no free ends apparent, were observed among the chromosomes. From contour-length measurements of several of these structures, assuming a 40:1 packing ratio of DNA¹⁴, a DNA content varying between 550 and 10³ kb was estimated. Figure 3 (a and b) shows two examples of such structures. These structures are not related to the aneuploidism of 10B3 cells, because we have not observed them in any of many aneuploid cell lines analysed. Although we cannot definitely equate the structures seen in the electron microscope with the 650-kb elements, the correspondence in size strongly suggests that they are related.

In the PFGE or field-inversion gel electrophoresis (FIGE) experiments discussed above, minor slowly-moving bands

Fig. 1 Pulse field gradient gel electrophoresis (PFGE) of genomic DNA isolated from different human cell lines, MTX-sensitive (HeLa S3) or MTX-resistant (HeLa BU25-10B3, VA₂-6A₂, VA₂-6A₃-B, VA₂-6A₃-E), or from *S. cerevisiae* (SS327), and detection of chromosomal structures containing amplified DHFR genes. *a* and *c*, EtBr-stained gels; *b*, DNA blot of the gel shown in *a* probed with the human DHFR cDNA clone pHD84². The gel shown in *a* and *b* was run with a switching interval of 90 s; that in *c*, with a switching interval of 60 s. The human cell variant VA₂-6A₂, which had been grown in 1.8×10^{-4} M DL-MTX for about one year, has been previously described¹; the cell lines VA₂-6A₃-B and VA₂-6A₃-E were derived by subjecting two samples of a precursor population of VA₂-6A₃ (ref. 1) at an early stage of the DHFR gene amplification process (resistant to 1×10^{-7} M MTX) to progressively increasing concentrations of the drug, up to 1.8×10^{-4} M, and subsequently maintained at this concentration for 11 and 7 months, respectively (unpublished data).

Methods. The cells were harvested by trypsinization, washed with phosphate-buffered saline (PBS) (without magnesium and calcium) at 37 °C, resuspended at 4×10^7 cells ml⁻¹, and gently mixed with an equal volume of a 1% solution of low-melting agarose (Seaplaque, FMC Bioproducts) in Mg²⁺, Ca²⁺-free PBS precooled to 37 °C. The agarose-cell mixture was allowed to solidify in the cold, and agarose-cell plugs were then treated with proteinase K (2 mg ml⁻¹) in the presence of 0.5% Sarkosyl, washed extensively, and finally treated with RNase A (100 µg ml⁻¹), under conditions to be described in detail elsewhere. After washing, the plugs were stored at room temperature in 10 mM Tris buffer, pH 8.0, 50 mM NaCl, 10 mM EDTA. Agarose plugs containing DNA from *S. cerevisiae* (SS327) were prepared as described by Schwartz and Cantor⁶. PFGE was carried out with a modification of the apparatus described by Carle and Olson⁷ (E. Lai, R. Barth & L. Hood, manuscript in preparation), using 1% agarose gels (SeaKem ME agarose, from FMC Bioproducts) in 0.5 × TBE buffer (TBE: 90 mM Tris borate, 90 mM boric acid, 2 mM EDTA, pH 8.0) and a 0.1-ml portion of the desired agarose-DNA plug (~20 µg DNA from the human cell lines). Electrophoresis was run at 10 V cm⁻¹ for 36 h (12–14 °C) with the switching time specified above, using recirculating 0.5 × TBE. After EtBr staining and UV visualization, the agarose gels were processed for DNA transfer by exposure to short-wave UV light for 60 s, soaking in 0.4 N NaOH for 15 min and blotting onto nylon membranes (Zeta-Probe, Biorad Laboratories) for 2 days in the presence of 0.4 M NaOH. After a brief exposure to 0.5 M Tris buffer, pH 8.0, 1.5 M NaCl, and baking in a vacuum oven, the filters were treated for hybridization with a ³²P-labelled probe according to standard procedures.



hybridizable with pHD84 were frequently observed in the genomic DNA samples from the 6A3-B and 6A3-E cell lines (see, for example, asterisked bands in Fig. 1*b*). To investigate the nature of these components further, the effect of varying the switching regimen on their mobility during FIGE was analysed. As shown in Fig. 4, by using the pHD84 insert instead of the whole plasmid as a probe, and thus reducing the background, one minor band (a) was clearly identified in the 10B3 DNA pattern, three (b', b'' and b''') in the 6A3-B pattern, and one (e) in the 6A3-E pattern. The mobility of the 650-kb band changed markedly depending on the switching interval, and consistently paralleled that of the yeast chromosomal DNAs of similar size (not shown). By contrast, the migration of the minor bands changed only slightly with the switching interval, suggesting that they represent supercoiled circular DNAs¹².

The results presented here indicate that, in MTX-resistant human cell lines, the extrachromosomal elements containing amplified DHFR genes can be much smaller than previously thought, so as not to be recognizable by optical microscopy. In a previous electron-microscopic analysis of the minute chromosomes from a mouse MTX-resistant cell line¹³, the smallest minute was estimated, by contour length measurements, to be $\approx 5 \times 10^3$ kb. From the EtBr staining patterns one can estimate that the 650-kb band deriving from ~20 µg of genomic DNA contains 50–100 ng of DNA. This is equivalent to 0.25–0.5% of the $\sim 10^{10}$ base pairs of the nuclear DNA of 10B3 cells, or to 40–80 elements per genome. If there is one DHFR gene per element, this would correspond to a significant fraction of the amplified genes detected in 10B3 cells (250–300 copies). The nature of the material in the wells containing amplified DHFR

Table 1 Chromosome constitution of MTX-resistant human cell lines

Cell line	Complement*	No. of chromosomes per cell†	No. of minutes per cell‡	Presence of HSR*
HeLa BU25-10B3	1S(95) 2S(5)	64 ± 1 93	6(0–12)	—
VA ₂ B-6A ₃ -B	1S(97) 2S(3)	109 ± 5 203; 214	209(50–566)	—
VA ₂ B-6A ₃ -E	1S(98) 2S(2)	104 ± 7 216	206(76–535)	—
VA ₂ B-6A ₂	1S(80) 2S(20)	56 ± 2 108 ± 2	150(14–482) 220(99–452)	+(97) +(100)

A total of 18 to 63 metaphase spreads were analysed from each cell line.

* Numbers in parentheses indicate the percentage of cells in each category. In HeLa BU25-10B3, the absence of HSR has been confirmed by *in situ* hybridization experiments. 1S and 2S indicate cells with the stem-line number of chromosomes and twice that number, respectively.

† Mean ± standard deviation. Whenever only one or two metaphases were found in a given group, the individual values are reported.

‡ Mean and range.

Fig. 2 *Sfi*I digestion of 650 kb elements carrying amplified DHFR genes from HeLa Bu25-10B3 cells and analysis of the products by FIGE. *a*, EtBr-stained gel; *b*, DNA blot of the gel shown in *a*, and *c*, DNA blot of another gel, both probed with nick-translated DNA from the extrachromosomal elements. The gel shown in *a* and *b* was run for 19 h, with a forward-migration interval varying between 10 and 65 s and a constant 3:1 ratio between forward and reverse interval; the gel shown in *c*, for 18 h, with a forward-migration interval varying between 10 and 51 s.

Methods. Several samples of HeLa BU25-10B3 genomic DNA were fractionated by FIGE on a 1% SeaKem GTG agarose (FMC Bioproducts) gel as described⁸, using a modified apparatus (E. Lai *et al.*, manuscript in preparation). Agarose plugs were excised from the region corresponding to the 650-kb elements of several adjacent lanes, and extensively washed with 10 mM Tris buffer, pH 8.0, 50 mM NaCl, 1 mM EDTA, at 4 °C. *a* and *b*, Two agarose-DNA slices were incubated for 3 h at 50 °C with 200 units of *Sfi*I (New England Biolabs) in 0.5 ml of buffer, and then for 2 h at 50 °C with proteinase K at 2 mg ml⁻¹. Two other agarose-DNA slices were treated in the same way, except for the absence of restriction enzyme. The two pairs of agarose-DNA slices, extensively washed with 0.5×TBE, and another agarose-DNA slice which had not been treated ('No inc.' lane) were embedded at the origin of a 1% SeaKem ME agarose slab, and subjected to FIGE in parallel with a sample of total genomic 10B3 DNA and with a bacteriophage λ DNA ladder (not shown). The split band in the samples incubated with or without *Sfi*I are due to mis-alignment and non-coherent migration of the two samples introduced into each well. After blotting the gel, the Zeta-Probe membrane was probed with nick-translated DNA from the extrachromosomal elements (electroeluted by FIGE). *c*, An agarose-DNA slice digested with *Sfi*I, as described above, was subjected to FIGE in parallel with a yeast DNA sample and with a λ ladder (not shown) for a shorter time than the samples in *a* and *b*.

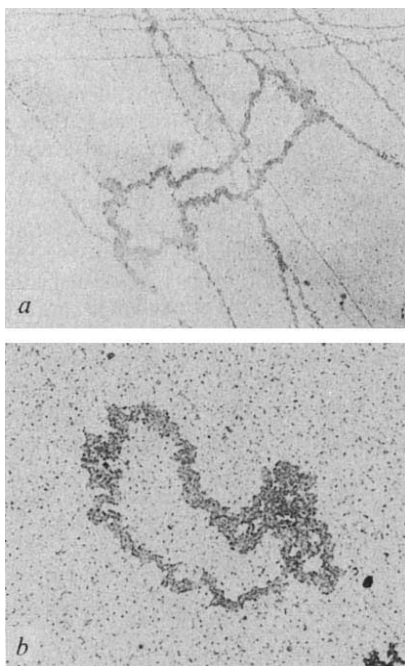
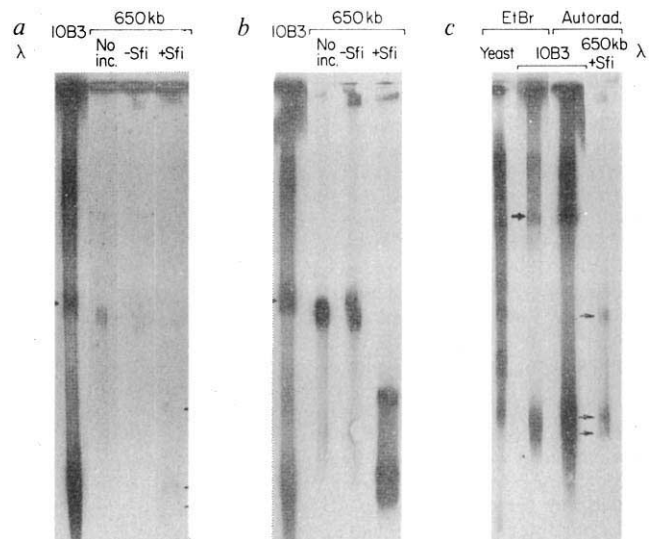


Fig. 3 Electron micrographs of extrachromosomal elements observed in 10B3 cells. *a*, ~580 kb, $\times 30,000$; *b*, ~710 kb, $\times 41,000$. The DNA contents were estimated from the contour lengths of the molecules, assuming a 40:1 packing ratio of DNA in the 20- to 30-nm chromatin fiber¹⁴.

Methods. Preparations were made from cells arrested in metaphase by incubation for 5 h in Colcemid (Gibco) at a final concentration of 90 ng ml⁻¹. After harvesting, the cells were pelleted, resuspended in 0.2 vol of medium and lysed by dilution with an equal volume of 1% NP40 (pH 10), and the lysate was deposited on an electron microscope grid as previously described¹³. After air-drying, the grids were stained with 1% phosphotungstic acid and viewed in a JEOL 100C electron microscope operated at 80 kV.

genes is not known, although trapping of the submicroscopic elements by the larger chromosomal DNAs or presence of oligomers of these elements could be important.

The structure of the 650-kb elements is still uncertain. The mobility of the 650-kb elements in PFGE and FIGE responds to changes in pulse times in a similar way to that of the linear chromosomal DNAs, and differently from that of small supercoiled circular DNAs. Furthermore, it is unlikely that the 650-kb elements are large relaxed circular DNA molecules, as it is

known that such molecules, like the 300–350-kb *Saccharomyces cerevisiae* K192 chromosome III DNA, are greatly retarded or even fail to enter an agarose gel (C. F. Hui, C. L. Smith, M. Mathew & C. Cantor, personal communication). Nothing, on the contrary, is known about the behaviour in PFGE or FIGE of large supercoiled DNA molecules. If the structures recognizable by E.M. are related to the 650-kb elements, as seems most likely, their apparent circularity may result from sticking of the ends of linear chromatin fibers; alternatively, the elements detec-

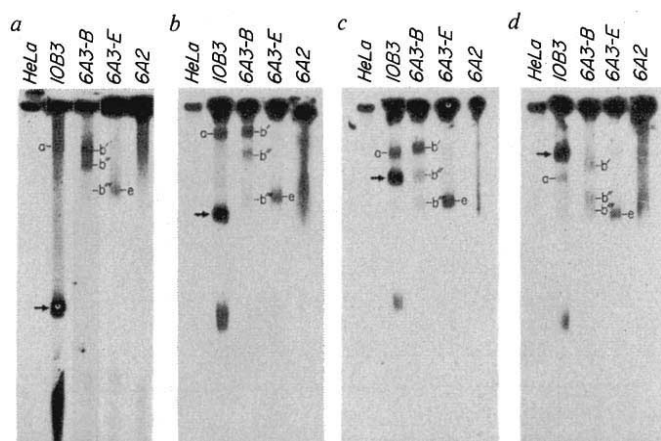


Fig. 4 Effect of changing the switching interval on the mobility in FIGE of the DHFR-gene containing extrachromosomal elements from 10B3, 6A₃-B and 6A₃-E cells. The human genomic DNA samples shown were run in FIGE using different switching regimens, as specified below; the gels were then blotted on Zeta-Probe membranes, and these were incubated with a nick-translated probe derived from the total pHD84 plasmid (a) or from the cDNA insert (b, c and d). The forward migration interval was 30–75 s in a, 30–80 s in b, 3–75 s in c, and 3–50 s in d; in all cases, a 3:1 ratio between forward and reverse intervals was used. The runs were carried out at 14 °C in a and at 11 °C in b, c and d.

ted by PFGE or FIGE may indeed be large supercoiled molecules. It is not known what relationship the minor types of DHFR gene-containing structures detected in 10B3, 6A₃-B and 6A₃-E cells have to the 650-kb elements.

The remarkable homogeneity in size of the 650-kb elements, which contrasts with the typical size variability of microscopically recognizable minute chromosomes, and their absence in other human MTX-resistant variants containing minute chromosomes suggests that they may arise by a mechanism distinct from that producing the latter structures. The occurrence of amplified DNA in 30-kb extrachromosomal circles of drug-resistant *Leishmania major* has recently been reported¹². The possibility of isolating in substantially pure form by PFGE or FIGE the 650-kb elements and the other extrachromosomal elements containing DHFR genes will make it possible to answer important questions concerning their sequence and gene organization, their expression, their capacity to replicate, and their role in the mechanism of gene amplification.

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Mutations in the active site of *Escherichia coli* phosphofructokinase

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The enzyme-catalysed transfer of a phosphoryl group from ATP is an important reaction in a wide variety of biological processes. We demonstrate here the essential function of an aspartate group in the catalysis of phosphoryl transfer by *Escherichia coli* phosphofructokinase, and the minor role of an arginine residue. We have used oligonucleotide-directed mutagenesis to replace two amino-acid residues which X-ray analysis has shown to be close to the transferred phosphoryl group and we have analysed the forward and back reactions of the mutant enzymes by steady-state kinetics. Changing Asp 127 to Ser reduced the turnover number by a factor of 18,000 in the forward direction and 3,100 in the back reaction, and the Michaelis constant for fructose 1,6-bisphosphate in the reverse reaction by a factor of 45. This shows that this aspartate is a key residue in the rate enhancement by the enzyme, probably acting as a base in the reaction mechanism, and that it also destabilizes the product complex. Changing Arg 171 to Ser reduced the turnover numbers by about 3.4, showing that this arginine has only a minor effect on the catalysis.

A great deal of work has been done to define the mechanism of enzyme-catalysed phosphoryl transfer from ATP and to identify the factors which enhance the reaction rate^{1–3}. Phosphoryl transfer reactions involve a nucleophilic attack of the acceptor molecule on the phosphoryl group. In enzyme-catalysed reactions this can proceed by either a direct, in-line attack, or the formation of a covalent phosphoenzyme intermediate^{3,4} and probably involves an associative mechanism in which a pentacoordinate transition state is formed³. The active site of a kinase could provide groups such as a general base to increase the nucleophilicity of the attacking nucleophile, electrophilic groups to stabilize the oxygens of the transferring phosphoryl group in the pentacoordinate transition state and a general acid to protonate the leaving group.

Phosphofructokinase (PFK) catalyses the phosphorylation of fructose 6-phosphate (F6P) by ATP to form fructose 1,6-bisphosphate (F1,6BP), ADP and a proton. This reaction is the first committed step in glycolysis and is important for metabolic control^{5,6}. The crystal structures of the PFKs from *Bacillus stearothermophilus*^{7,8} and *E. coli* (Y. Shirakihara, W. R. Rypniewski and P.R.E., unpublished) have been determined. These two enzymes, very similar in sequence⁹ and structure, are homologous to the rabbit muscle PFK which appears to have evolved from a duplication of the bacterial genes¹⁰. The reaction catalysed by the *B. stearothermophilus* and rabbit muscle enzymes has been shown to invert the configuration of the transferred phosphoryl group¹¹, which, together with the structure of the active site F6P-MgADP ternary complex of the bacterial PFKs^{7,8}, suggests a direct, in-line nucleophilic attack of the 1-hydroxyl of F6P on the γ -phosphoryl of ATP (Fig. 1). The structure of the active site also suggests that the carboxyl group of Asp 127, which is conserved in all PFK sequences^{9,10}, acts as the general base to increase the nucleophilicity of the 1-hydroxyl. Finally, a model of a pentacoordinate γ -phosphoryl group placed in the position which it might occupy in the active site showed that the guanidinium group of Arg 171, which is also conserved^{9,10}, could form hydrogen bonds with one of the planar oxygens in the trigonal bipyramid (Fig. 1). Mutations in

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Table 1 Steady-state rate analysis of wild-type and mutant PFKs

Parameter	Direction of synthesis	Wild type	Arg → Ser 171	Asp → Ser 127	Ser 171 and Ser 127
$k_{\text{cat}}(\text{s}^{-1})$	F1,6BP	93	27	5.2×10^{-3}	1.5×10^{-3}
	F6P	2.3	0.70	0.75×10^{-3}	ND
k_{cat} (relative to wild-type)	F1,6BP	1	1/3.4	1/18,000	1/62,000
	F6P	1	1/3.3	1/3,100	ND
$K_m^{\text{f}}(\text{ATP}) (\mu\text{M})$	F1,6BP	41	33	31	14.5
$K_m^{\text{f}}(\text{F6P}) (\mu\text{M})$		38	34	33	ND
$K_m^{\text{b}}(\text{ADP}) (\mu\text{M})$	F6P	32	21	9	ND
$K_m^{\text{b}}(\text{FDP}) (\mu\text{M})$		360	350	8	ND

ND, not determined. All enzyme assays were done with saturating amounts (1 mM) of the activator GDP so that the kinetics showed a hyperbolic rather than a sigmoidal variation with respect to F6P concentration²⁰. The K_m values of one substrate were determined at saturating concentrations (1 mM) of the other. Enzyme concentrations for determining k_{cat} values were deduced from measuring the absorbance at 278 nm (specific absorbance of PFK is $\epsilon_{278} = 0.60 \text{ cm}^3 \text{ mg}^{-1}$ (ref. 21)) and the subunit relative molecular mass, 34,817 was calculated from the amino-acid sequence⁹. The reaction was assayed in the direction of F1,6BP synthesis by coupling F1,6BP production to the oxidation of NADH using aldolase ($20 \mu\text{g ml}^{-1}$), triose phosphate isomerase ($10 \mu\text{g ml}^{-1}$) and glyceraldehyde-3-phosphate dehydrogenase ($10 \mu\text{g ml}^{-1}$). ADP was regenerated to ATP using creatine phosphate (1 mM) and creatine kinase ($10 \mu\text{g ml}^{-1}$). The back reaction was assayed by coupling the production of F6P to the reduction of NADP using glucose-6-phosphate isomerase ($10 \mu\text{g ml}^{-1}$) and glucose-6-phosphate dehydrogenase ($10 \mu\text{g ml}^{-1}$). ATP was regenerated to ADP using glycerol (1 mM) and glycerol kinase ($20 \mu\text{g ml}^{-1}$). All enzymes and substrates were from Boehringer Mannheim. All enzyme assays were done at 25 °C, pH 7.9 in 100 mM Tris-HCl, 10 mM NH_4Cl , 10 mM MgCl_2 , using a thermostatted Gilford 410 spectrophotometer to monitor the change in absorbance at 340 nm due to the change in the oxidation state of NADH or NADP. Wild-type and mutant PFKs were prepared from an *E. coli* strain, DF1020, deleted for both PFK genes, transformed with pUC9 recombinants of the constitutively expressing mutant genes⁹ and purified by loading a cleared lysate from such a strain on a BlueF3GA-Agarose column (Matrex Blue A, Amicon), which was washed with 100 mM Tris-HCl (pH 8.0), 1.5 M NaCl, 1 mM β -mercaptoethanol, after which PFK was eluted by washing with 5 mM ATP (Sigma), 10 mM MgCl_2 , 1 mM DTT. This eluate was concentrated and stored as a 55% suspension in $(\text{NH}_4)_2\text{SO}_4$ at 4 °C. For enzyme assays, this suspension was desalted on a Sephacryl-200 column (Pharmacia). The pH profiles of k_{cat} of the mutants were distinctly different from the wild-type, indicating that the mutant proteins were not contaminated with significant amounts of wild-type enzyme (data not shown). Oligonucleotides for mutagenesis were synthesized on an automated DNA synthesizer (Biolabs Sam-One) and used to introduce mutations into the M13mp8 recombinant mHE1011 (refs 9, 22, 23).

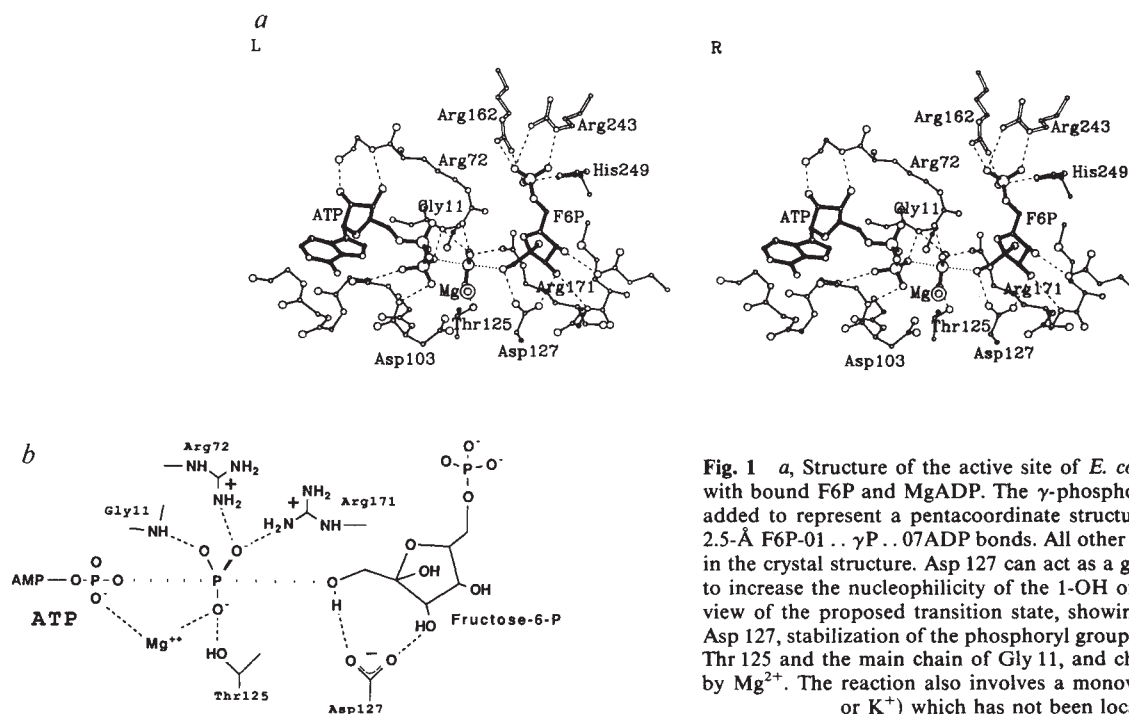


Fig. 1 *a*, Structure of the active site of *E. coli* PFK determined with bound F6P and MgADP. The γ -phosphoryl group has been added to represent a pentacoordinate structure with equidistant 2.5-Å F6P-O1... γ P...O7ADP bonds. All other atoms are shown as in the crystal structure. Asp 127 can act as a general base catalyst to increase the nucleophilicity of the 1-OH of F6P. *b*, Schematic view of the proposed transition state, showing base catalysis by Asp 127, stabilization of the phosphoryl group by Arg 171, Arg 72, Thr 125 and the main chain of Gly 11, and charge neutralization by Mg^{2+} . The reaction also involves a monovalent cation (NH_4^+ or K^+) which has not been located.

these two residues can therefore be expected to perturb the catalytic mechanism of the enzyme.

Using oligonucleotide-directed mutagenesis¹², the codons for the Asp 127 and Arg 171 were each changed to serine in the *E. coli* PFK gene which had previously been cloned, sequenced and expressed to high levels⁹. The steady-state kinetic parameters k_{cat} (turnover number) and K_m (Michaelis constant) were determined for the forward and reverse reactions for wild-

type and mutant enzymes (Table 1), in the presence of the activator GDP to eliminate the cooperative behaviour of the enzyme. These parameters are composites of the rate constants of individual steps of the reaction, which may be most simply divided into three stages: binding of substrates, the chemical conversion step and the release of products (Fig. 2). Ideally, we would like to know the effect of the mutations on these individual rate constants, which would require pre-steady-state kinetic

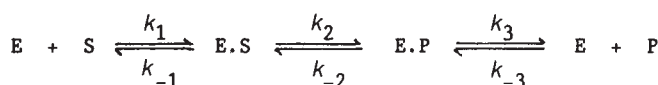


Fig. 2 Simplified reaction scheme (E, PFK; S, ATP and F6P; P, ADP and F1,6BP). It can be shown that in the forward reaction assuming one substrate is saturating, the rate equation with respect to the other substrate has the following composite parameters (see for example ref. 19)) $k_{\text{cat}}^f = k_2 k_3 / (k_2 + k_{-2} + k_3)$ and $K_m^f = K_s^f (k_3 + k_{-2} + k_2 k_3 / k_{-1}) / (k_2 + k_{-2} + k_3)$, where $K_s^f = k_{-1} / k_1$ and corresponds to the dissociation constant for E.S. Of these terms, only k_1 and k_{-1} (and hence K_s^f) are different for the two substrates F6P and ATP. Similarly, for the back reaction $k_{\text{cat}}^b = k_2 k_{-1} / (k_2 + k_{-2} + k_{-1})$ and $K_m^b = K_s^b (k_{-1} + k_2 + k_{-2} k_{-1} / k_3) / (k_2 + k_{-2} + k_{-1})$, where $K_s^b = k_3 / k_{-3}$. Constants k_3 , k_{-3} and K_s^b are different for F1,6BP and ADP.

measurements. Nevertheless, by considering the changes in k_{cat} and K_m caused by the mutations, we can assign the changes to the different stages of the reaction, even if we cannot quantify the effect on the rate constants.

The Ser 127 mutant has greatly diminished k_{cat} values in both directions of the reaction (Table 1). Much of this large change can be ascribed to the catalytic step (k_2 and k_{-2} in Fig. 2), because the rate for the mutant enzyme is too low for rate-limiting product release (k_3 , which might also contribute to k_{cat}). The much smaller decrease in k_{cat} caused by the Arg → Ser 171 mutation is probably also due to changes in the catalytic rates k_2 and k_{-2} , because the changes in K_m^f , the Michaelis constants for the forward reaction, are small and similar for the two substrates.

Values of K_m^f are changed only a little by either mutation and by about the same amount for the two substrates F6P and ATP. This is most simply ascribed to a change in k_2 , with little change in the dissociation constants K_s^f for the two substrates, because it is unlikely that a mutation would affect K_s^f equally for both substrates. On the other hand, the K_m^b values for the reverse reaction change by different amounts: K_m^b (F1,6BP) is reduced by a factor of 45 in the Ser 127 mutant, whereas K_m^b (ADP) is reduced by less than fourfold. This suggests that the removal of the Asp 127 carboxyl group in this mutant has stabilized the complex E.P in Fig. 2 and reduced the dissociation constant K_s^b (F1,6BP).

The large changes in k_{cat} when the carboxyl group of Asp 127 is removed are probably due to its acting as a general base in the forward reaction and as a general acid in the reverse reaction. Such a mechanism has been suggested, for example, for hexokinase^{13,14} and staphylococcal nuclease¹⁵. General acid catalysis has also been demonstrated in the hydrolysis of salicyl phosphate¹⁶. We cannot exclude the possibility that the carboxyl group acts simply to orientate the accepting nucleophile correctly, but significant structural changes are unlikely, because the changes in K_m are small. This aspartate is not (in the ADP complex) close to the Mg^{2+} ion, which probably binds to Asp 103, so the effect of the mutation is unlikely to be due to changes in Mg^{2+} binding. The change in K_m^b (F1,6BP) shows that the Asp 127 side chain also destabilizes the E.F1,6BP.ADP product complex (E.P in Fig. 2), presumably by repulsion between the negatively charged carboxyl and the newly formed 1-phosphate on the sugar, thus aiding the release of products. It is not possible from these data to know whether in the wild-type enzyme the chemical step or product release is rate limiting in the forward reaction, but the presence of the Asp 127 side chain speeds up both steps.

Contrary to our expectations, Arg 171 does not appear to be of major importance in stabilizing the transition state. The evidence from non-enzymatic catalysis on the importance of such electrophilic catalysis is equivocal^{17,18}. In many model systems, phosphoryl transfer seems to occur by a dissociative

mechanism (forming a metaphosphate ion), but in an enzyme the distinction between associative and dissociative mechanisms is less clear, because the accepting nucleophile needs to be in place before the phosphoryl group leaves ATP (ref. 3), and the observed inversion of the phosphoryl group would seem to favour an associative pathway. Positively charged groups near the phosphoryl group would be expected to slow down a dissociative pathway, but speed up an associative pathway. The small catalytic effect we see here from Arg 171 is insufficient to make a distinction between these alternatives. Other positively charged residues in the active site near the γ -phosphoryl group, such as Arg 72, may prove important for this aspect of the catalytic mechanism. Further mutations will have to be constructed to investigate this.

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Erratum

Isolation of an excision product of T-cell receptor α -chain gene rearrangements

Shinji Fujimoto & Hideo Yamagishi

Nature **327**, 242 (1987).

Figure 1 was not printed, it appears here with its legend.

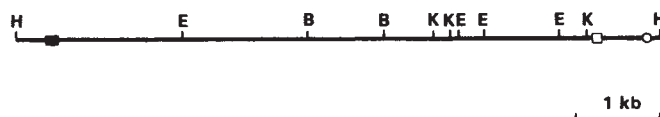


Fig. 1 Restriction of a p113 spc-DNA insert containing the fragment rearranged for the genomic sequence. Restriction sites are indicated by vertical lines above the horizontal line. Restriction enzymes: B, *Bam*HI; E, *Eco*RI; H, *Hind*III; K, *Kpn*I. The location of a short interspersed repetitive B1 sequence (■) was determined by Southern blot analyses and the locations of J_1 (□) and the 'reciprocal joint' (○) were determined by sequencing the 0.85-kb *Kpn*I–*Hind*III fragment.

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Supercritical fluid chromatography

M. L. Lee and K. E. Markides

Supercritical fluid chromatography is opening up new avenues in the analysis of high molecular weight, polar and thermally labile biochemicals.

SUPERCritical fluid chromatography (SFC) is a column chromatographic technique in which the mobile phase is a supercritical fluid instead of a gas or liquid, as in gas or liquid chromatography. The stationary phase can be either a solid adsorbent, as in adsorption chromatography, or a liquid, as in partition chromatography. SFC was first reported in 1962 by Klesper and coworkers¹, who used supercritical chlorofluorocarbons to separate metal porphyrins. This first report generated considerable interest among chromatographers, but a lack of understanding of the physicochemical properties of supercritical fluids, and the stringent demands on instrumentation imposed by the high pressures required to transform gases or liquids into supercritical fluid mobile phases, limited the further development of the method. The recent dramatic rise in the popularity of SFC is primarily due to improvements in the packed-column approach², the introduction of capillary column SFC in 1981³, and the commercialization of SFC instrumentation within the last two years.

While by strict definition there are only three states of matter — solid, liquid and gas — supercritical fluids possess unique properties. They usually have densities, viscosities, and other physical properties that are between those of gases and liquids. The critical temperature of a substance is defined as the temperature above which it cannot be condensed into a liquid, regardless of the amount of pressure that is applied. Supercritical fluids can be likened most to "dense" gases, but densities and intermolecular interactions sufficient to impart a solvating effect on solute molecules for chromatographic migration differentiate them from typical dense gases. For these reasons, SFC can achieve higher efficiencies in shorter analysis times than liquid chromatography, and can perform the separation of thermally labile and nonvolatile solute samples that cannot be studied using gas chromatography. Figure 1, for example, demonstrates a separation of steroid glucuronides isolated from human urine using carbon dioxide as the mobile phase⁴. Similar results were achieved for glycine and taurine conjugates of bile acids in their intact forms.

Tools of the trade

The basic instrumental components used for SFC are adapted from those used in either LC or GC. A high-pressure liquid delivery pumping system is the mobile

phase source, and is controlled by a microprocessor to give precise and accurate pressure control and density programming. Because fluid density is the major factor influencing solute solubility, and hence chromatographic retention, density programming can be applied to SFC in the same manner that temperature and solvent programming are used in GC and LC⁵ respectively.

The sample introduction devices for SFC are either high pressure sampling valves or extraction cells. Using sampling valves, a sample usually is introduced as a liquid solution in an appropriate solvent. With an extraction cell, the analytes of interest are extracted directly from the sample matrix (soil, plant material, food, tissue, etc.), concentrated, and introduced immediately into the chromatographic system. This simplifies and shortens the time spent in sample preparation, and increases sensitivity.

The analytical column is placed in a chromatographic oven which can be maintained isothermally or programmed above the critical temperature of the mobile phase. The end of the column can be interfaced to a number of detectors, the most popular being the flame ionization detector (FID) and the UV-absorption detector, or to spectroscopic detection systems such as mass spectrometers or Fourier transform infrared spectrometers⁶.

Chromatographic columns

The two types of columns available for use in SFC are packed columns and open-tubular (capillary) columns, as is the case for both GC and LC. For packed column SFC, typical LC columns (4.6, 2.0 and 1.0 mm i.d.) that contain small particle packings (3, 5 and 10 μm in diameter) can be used. Such columns can produce 8,000 to 10,000 total theoretical plates. On the other hand, columns of smaller diameter (25–80 μm i.d.) achieve higher efficiencies (> 3,000 plates per metre) in shorter analysis times (< 1 hour)⁷. Capillary columns are generally used in lengths of between 1 and 25 m, and produce better separations of complex mixtures than do packed columns. For preparative-scale separations, however, where the objective is sample collection, packed columns are essential.

Detector compatibility and sample capacity also should be considered when selecting an analytical column. Mobile phase flow rates can be up to 20 ml min⁻¹ for packed columns — much higher than the capillary column flow rates of 1–10 μl

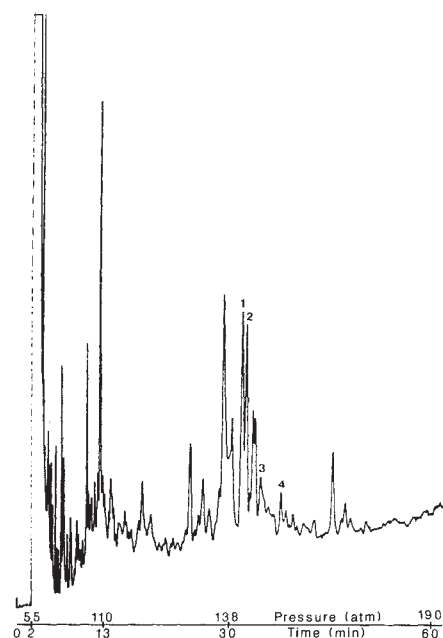


Fig. 1 Capillary supercritical fluid chromatogram representing the glucuronide fraction isolated from human urine. Conditions: supercritical CO₂ mobile phase at 95° C, 10 m × 50 μm i.d. SE-30 (d_f = 0.25 μm) fused silica column. Peak 1, pregnandiol-glucuronide, 2, 11 β -hydroxyetiocholanolone-glucuronide, 3, androsterone-glucuronide, 4, epiandrosterone-glucuronide.

min⁻¹. The high flow rate of packed columns prohibits their use with many types of detectors without splitting, but splitting the column effluent before detection greatly reduces sensitivity.

Applications

Interestingly, during its initial development, SFC was applied mainly to the analysis of polymers, polymer additives, petrochemicals, agrichemicals, surfactants, dyes, and other industrial chemicals. Many of these chemicals are either thermally labile, do not possess chromophores for easy detection using common LC detectors, or are of high molecular weight, and therefore nonvolatile. More recently, the technique has generated considerable interest for use in the analysis of drugs, pharmaceuticals and their metabolites. This is an obvious extension of the technique because biomolecules are often large, nonvolatile, thermally labile, and polar with a wide variety of functional groups.

Specific examples of biological compounds that have been analysed by SFC include fatty acids⁸, prostaglandins⁸, triglycerides⁹, archaeobacterial lipids¹⁰,

trichothecene mycotoxins¹¹, polysaccharides¹², steroids¹³, drugs of abuse and therapeutic drugs¹³. In most of these cases, supercritical carbon dioxide was used as the mobile phase. Carbon dioxide is popular because it has a low critical temperature (32° C), is chemically inert, and can be used with most detection systems, most importantly the FID and mass spectrometer. Carbon dioxide is relatively nonpolar, but quite polar solute molecules can be chromatographed when extremely low surface area, well-deactivated capillary columns coated with nonpolar stationary phases are used.

However, the application of SFC to significantly polar biomolecules necessitates the use of more polar mobile phases. Unfortunately, most polar substances have high critical temperatures or are too reactive to be used in chromatography. One exception is ammonia, which has recently shown promise in the analysis of polar basic compounds¹⁴. Mobile phases may be tailored by adding polar modifiers, and considerable effort is currently being concentrated in this direction. Modifiers such as methanol, isopropanol, acetonitrile, methylene chloride, tetrahydrofuran, dioxane, dimethylformamide, propylene carbonate, and formic acid have been shown to increase the solvent power of superficial carbon dioxide significantly.

Recent promising results using supercritical mobile phases containing reverse micelles with aqueous centre pools indicate that reverse micelles may offer an alternative to polar and modified fluids¹⁵. Such mobile phases are capable of solvating large molecules such as cytochrome c in nonpolar supercritical fluids¹⁶. □

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What's new at the ASBC

Next week's meeting of the American Society of Biological Chemists in Philadelphia, Pennsylvania, will have over 200 exhibits. Here we preview some of the products on view.

THE American Type Culture Collection has released its **workshop schedule** for the second half of 1987 (Reader Service No. 101.) The emphasis at ATCC in June through December will be on freezing and freeze-drying of microorganisms and cell cultures, recombinant DNA, and hybridomas and monoclonal antibodies. Additional workshops will focus on clinical anaerobic bacteriology, fermentation microbiology, and hybridoma data management. Costs for the hands-on workshops (held at ATCC facilities in Rockville, Maryland) vary, but staff on the ATCC booth, number 743, will be ready to answer questions and offer brochures.

Miles Diagnostics, exhibiting in booth 751, has written the book on **biochemicals for cell culture**. It has just released a free catalogue entitled *Partners in Progress* that outlines its range of human, rabbit and bovine albumins, lipoproteins, transferrins, coagulation factors, globulins and



Miles has an application guide to accompany its catalogue.

bovine and human sera (Reader Service No. 102). The catalogue also includes details on Ex-cyte, the growth enhancement media supplement rich in cholesterol, phospholipids and fatty acids that cause the modulation of cell membrane proteins and increase antibody secretion. The 32-page booklet is accompanied by a brochure called *The Perfect Fit* that contains charts to enable researchers to match Miles' products to their own specific applications.

DNA details

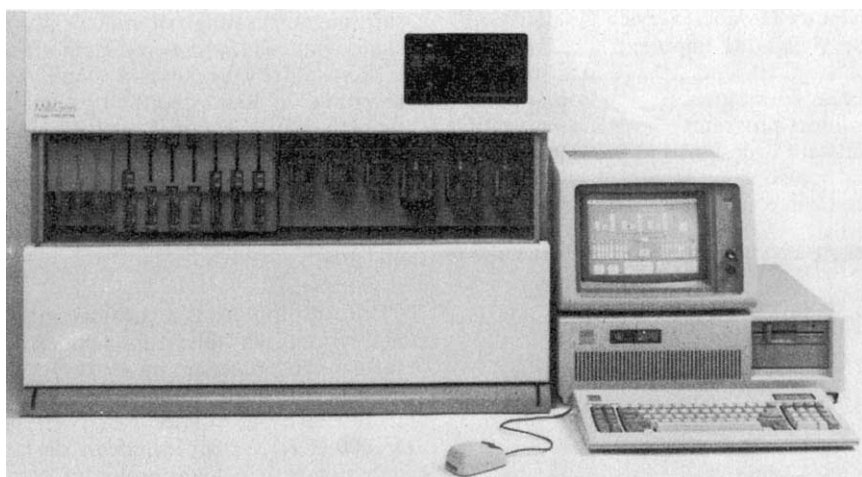
Traditional blotting techniques may be a thing of the past, now that NuFix **glyoxyl agarose** from FMC (Marine Colloids) is available from ICN Biomedicals, exhibiting in booths 277 and 279 (Reader Service

No. 103). ICN says NuFix is a revolutionary new tool for electrophoresis, because it allows proteins and polypeptides to be separated and immobilized still in the gel in their non-denatured, biochemically reactive state. Samples can be fixed reversibly to the agarose matrix by increasing and decreasing the pH of the buffer, or be bound permanently with the addition of cyanoborohydride to the alkaline buffer. Additional uses for NuFix, sold in 25g packages for £62 (UK), include screening of hybridomas, or as a preactivated chromatography medium. As an introductory offer, ICN is giving away 10 free sheets of 85×100 mm GelBond for casting gels with a first purchase of NuFix agarose.

Clontech, in booth 567, has just announced its GUS Gene Fusion System, that uses the enzyme beta-glucuronidase (GUS) as a **gene fusion marker** for the analysis of gene expression (Reader Service No. 104). Clontech says the marker is most useful in plants, yeast or *Drosophila* because they lack intrinsic beta-glucuronidase activity. Successful fusion attempts are revealed by the enzyme's activity, as assayed by spectrophotometric, fluorometric and histochemical methods. The \$295 (US) GUS kit contains plasmids, GUS substrate, X-glucuronide, an oligonucleotide for sequencing, GUS enzyme, GUS antibody, and an instruction manual.

New England BioLabs has added five new members to its list of over 100 **restriction endonucleases** and nucleic acid modifying enzymes (Reader Service No. 105). Four of the new enzymes are only available from New England BioLabs, according to the company: *AlwI* (cleaves GGATC(4/5)), *AlwNI* (cuts CAGNNN/CTG), *BspHI* (cleaves T/CATGA), and *PleI* (recognizes GAGTC(4/5)). The fifth, *FspII*, cuts TT/CGAA. The restriction enzymes are sold in 20 to 250 unit samples. New England BioLabs has also announced price reductions for *BanI*, *Bsp1286*, *OxaNI*, *SalI*, *SphI*, *XbaI*, *XhoII* and T4 RNA ligase that range from 20 per cent to 10-fold. More information on New England BioLabs' enzymes can be found in booths 560 and 562.

Millipore's bioinstrumentation division, MilliGen, will be showing off its new **DNA synthesizer** in booths 712, 714 and 716 (Reader Service No. 106). MilliGen says the model 7500 synthesizer has a



Millipore's gene machine uses a popular DNA synthesis chemistry, and is backed by a guarantee.

greater than 99 per cent coupling efficiency, cycle times of four minutes per step, and low reagent consumption. MilliGen's \$42,500 (US) gene machine uses easy-on-the-nose beta-cyanoethyl phosphoramidite chemistry, and takes instructions from any IBM PC or compatible. The system includes MilliGen Express-DNA software, and features an Auto-Amidite anhydrous mixing device to conserve costly amidites. MilliGen says the synthesizer is a practical system for making and calibrates the instrument at an operator-selected date and time. Perkin-MilliGen will synthesize a 100-base sequence as a final check that the system is operating at peak performance.

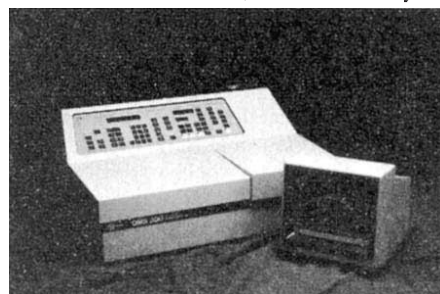
Smart analysers

Sargent-Welch, displaying in booths 866 and 868, has a new **titrator** designed to automate, accelerate and simplify titrimetric procedures without compromising precision or accuracy (Reader Service No. 107). The model MPT II Titrator has a microprocessor in charge, and standard features include non-volatile program storage, English language prompting, normal and differential data presentation,

automatic and/or manual plot expansion, automatic endpoint identification, calculation of sample concentration, per cent weight and electrode efficiency, multiple buret control and multisample control. It functions as an independent unit, but is also compatible with Lotus 1-2-3 Measure and Labtech Notebook data acquisition software, to allow for full PC integration. The MPT II performs preset, recorded and incremental pH titration and mv titration, back titration (pH and mv), pH stat, pH and temperature readout, mv and temperature readout, pre-delivery of titrant and precise liquid dispensing. Sargent-Welch has priced its titrator at \$8,600 (US) for the basic instrument, or the "loaded" version can be had for \$13,698 (US).

Varian, in booths 772-779, has expanded its range of **routine UV-vis spectrophotometers** with the addition of the DMS 300 model, a double-beam instrument with optical grating (Reader Service No. 108). The DMS 300 uses the same plug-in application modules made popular by earlier models, that eliminate time-consuming manual transfer, entry and interpretation

of data. The modules provide application-specific soft-key menus from which researchers can establish data and mathematical routines for calculating final results online for scanning, kinetics and quantitative analysis. Once the application programs have been developed, they can be called up for future use with the touch of a key. Varian has geared its spectrophotometer toward researchers who like the high-performance characteristics of the finest instruments, but who don't have enough cash. For \$13,500 (US), Varian says the DMS 300 makes accurate measurements above 5 absorbance, and can handle highly concentrated, minimum dilution samples. Kinetic capabilities include slope and activity calculations for zero and first-order rate reactions for up to five cells, slope window calculations on user-defined windows, and the ability to



Another module-based spectrophotometer from Varian.

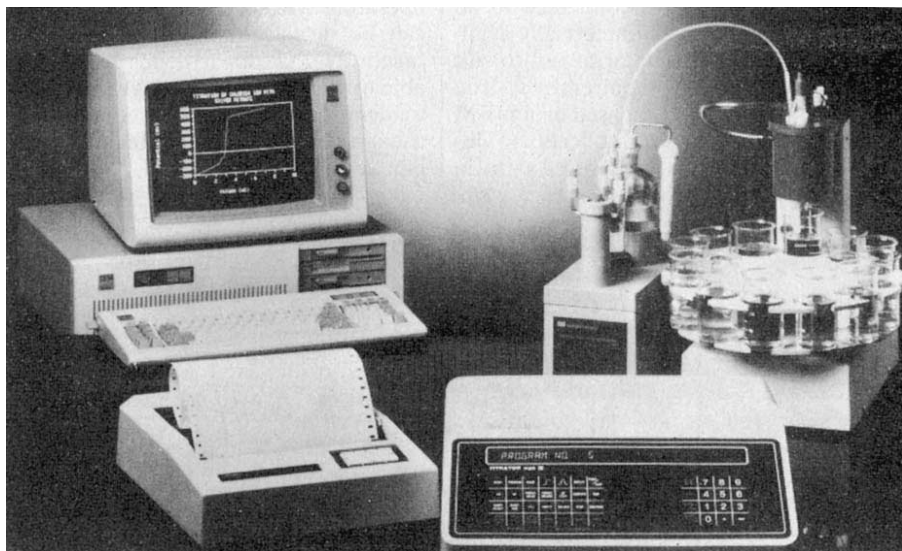
average slopes. Built-in concentration functions include calibration for up to ten standards and five replicates in a choice of photometric modes, choice of curve fits, weight correction, and identification of out-of-range sample concentrations.

Busy labs might benefit from the Tri-Carb 2200CA **liquid scintillation analyser**



High-volume liquid scintillation counts from Packard.

from Packard Instrument Company, that holds 408 standard or 720 small vials (Reader Service No. 109). At its heart is an IBM-compatible PICO-ATE computer that supports tandem processing of applications programs, commercial software and user-written routings. The Tri-Carb's automatic instrument performance assessment and self-calibration features monitor backgrounds, efficiencies, E^2/B , and Chi-square values for ^3H and ^{14}C counting, and even reports impending problems. The spectral unfolding function divides the composite spectrum of dual label samples into a 3-dimensional colour



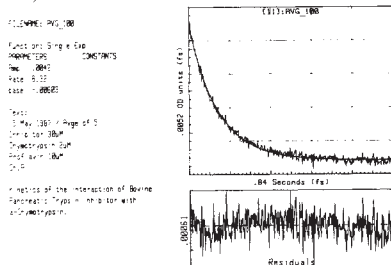
Sargent-Welch has just the machine to take the tedium out of titrating.

plot and displays the c.p.m. and d.p.m. calculations of both radionuclides. Efficiency tracing determines absolute activities without quench correction, and Packard says it is not sensitive to colour quenching, volume variations or wall effect. The \$24,200 (US) Tri-Carb 2200CA offers six d.p.m. calculation methods, including one that calculates d.p.m. in three regions of interest simultaneously, and has factory-stored quench curves for single and dual label counting of ^3H and ^{14}C . Packard Instrument Company will be in booths 655, 657, 754 and 756.

Automatic measurement of the carbon, hydrogen and nitrogen content of organic materials is the specialty of Perkin Elmer's PE 2400 CHN Elemental Analyzer (Reader Service No. 110). The \$24,000 (US) PE 2400 is based upon classical **elemental analysis** techniques, and burns samples in a pure oxygen environment to ensure complete combustion. According to Perkin Elmer, analysis time is five minutes, and a 60-position autosampler is available for automatic unattended operation. The microprocessor brain behind the PE 2400 eliminates time wasted waiting around for the machine to warm up by controlling a wake-up function that equilibrates and calibrates the instrument at an operator-selected date and time. Perkin-Elmer will be displaying its self-starter in booths 449-556.

Software packages

Hi-Tech Scientific, exhibiting in booth 860, has launched a data acquisition and processing package for the Hewlett-Packard Bobcat 300 Series technical desktop computers (Reader Service No. 111). The HS-1 Technical DataPRO Software Suite is meant for **solution kinetics studies**, and interfaces with most commer-

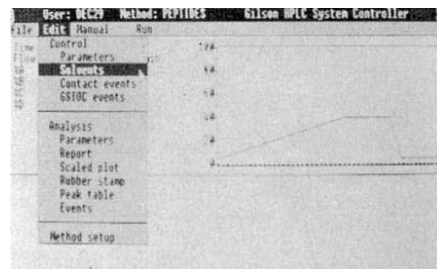


The Hi-Tech software for solution kinetics studies.

cially available rapid kinetic spectrometers including the 90 nm msec⁻¹ Hi-Tech SpectraScan. Hi-Tech says the SpectraScan can record ageing times over a range of milliseconds to hours, and maintains sample solutions under thermostatted, anaerobic and chemically inert conditions. The DataPRO software sells for £2,780 (UK), and the SpectraScan has a £6,850 (UK) price tag.

Gilson Medical Electronics has recently upgraded its 714 **HPLC system controller**

software (Reader Service No. 112). Gilson claims the improved software offers faster HPLC operations and affords users access to resident word processing and graphics programs. The IBM-compatible software is designed to provide researchers access to on-screen chromatograms, gradient control and data analysis, and



New software gives Gilson HPLC new abilities.

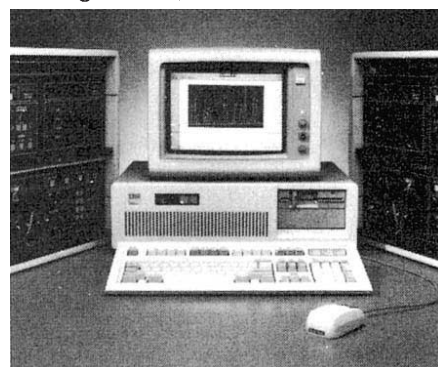
give them the capability to alter any parameter instantly. The software gives users control of up to four Gilson pumps and peripherals such as auto-injectors and UV detectors. Four channels of information may be manipulated, saved, analysed and plotted using the software, applying different parameters to each channel for optimal analysis. Gilson offers the software separately for \$2,200 (US), or as an integrated hardware/software package consisting of Gilson HPLC components and an IBM PC AT, starting at \$18,000 (US). Systems are configured according to user's needs, based on choices of separation quantity, mode of mobile phase delivery and mode of injection. Gilson will have a display in booths 424-427.

HPLC goes modular

The System 400 from Kontron Instruments, exhibiting in booth 737, offers a **modular approach to HPLC** (Reader Service No. 113). Each component of the system has its own RS-232 interface, allowing them to be used together in various configurations, or as stand-alone units. All modules are connected to an RS-multiplexer using commercially available cables, and can be arranged to suit specific applications requirements. The central data system 450, based on an IBM AT/XT-compatible PC, collects, evaluates, stores and plots measured data in

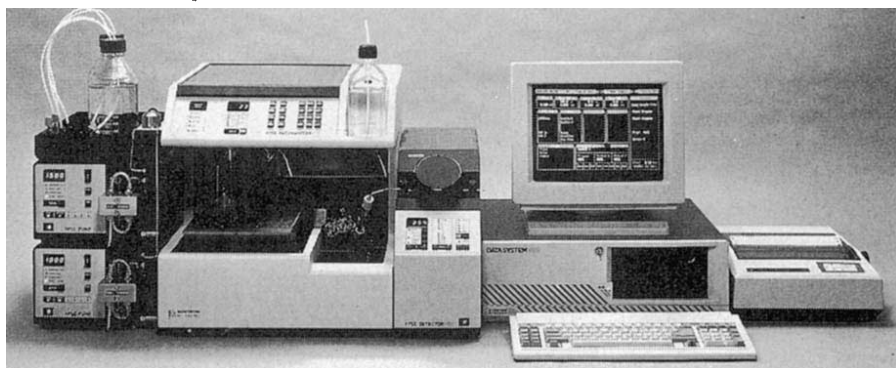
real time. Operating parameters can be set automatically, and stored methods can be executed by pressing a single key. According to Kontron, the system has completely new routings that eliminate the need for the definition of intergration parameters, although zooming of sections of a chromatogram, alteration of peak limits and baseline correction are still available.

The AutoIon 400 is a chromatography data system available from Dionex Ltd that can **link together up to twelve ion chromatographs**, regardless of the manufacturer (Reader Service No. 114). The £16,800 (UK) system includes a desktop computer with a high-resolution colour monitor, a 30 MByte hard disk, a printer-plotter and a Dionex buffered computer interface module (CIM). Each CIM controls a dual-system ion chromatograph with up to two detectors, as well as accessories such as autosamplers, sampling pumps, or column switching valves. The CIM converts analogue detector signals into digital data, and stores the data in its



Dionex networks ion chromatography with the AutoIon 400.

128K RAM memory until each chromatogram is complete, minimizing the load on the central computer, and freeing it for other tasks. The AutoIon 400 software uses a multi-tasking MS-DOS Windows operating system, so several applications can be displayed on the screen simultaneously. Pull-down menus enable available options to be viewed without having to memorize strings of key sequences. For users who already own an IBM PC or 100 per cent compatible, the AutoIon 400



The Kontron Instruments system for HPLC is composed of interchangeable components.

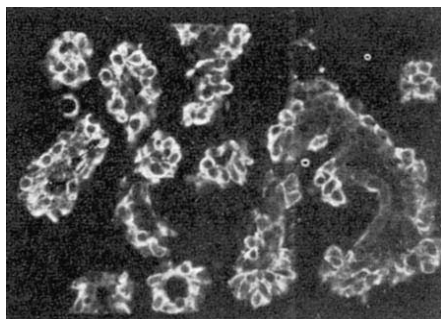
software with one CIM is sold for £12,000 (UK). More information on Dionex's products can be found in booths 555 and 557.

Beckman Instruments' new System Gold series of Personal Chromatographs integrate **personal computers with liquid chromatography modules** for solvent delivery, detection and data handling in configurations to handle a variety of specific applications (Reader Service No. 115). Designed particularly for life science research, the Personal Chromatographs can be preconfigured for methods development, metabolism detection with online radioisotope detection, semipreparative LC, amino acid analysis, nucleic acid analysis, protein isolations and peptide separations. System Gold set-ups are offered with either a desktop IBM PC, that can control two HPLC systems of up to eight LC modules each, or a NEC lap-top computer that monitors one eight-module system. The IBM-based System Gold chromatographs employ a mouse, and provide data collection and multiple automated analyses with interactive graphics. The chromatographs controlled by the NEC lap-top are geared toward applications where data quantitation is not required, and chromatographs need to be linked to LIMS networks for central data archiving and analysis. Beckman will have more details about System Gold in booths from 313 to 520.

The line on immunoreagents

Porton Products Ltd, in booths 561, 563 and 565 has developed a **recombinant form of Protein A** (Reader Service No. 116). Porton's Protein A is expressed in an *Escherichia coli* host, eliminating the toxin problems associated with some *Staphylococcus aureus*-expressed versions. The recombinant Protein A lacks a C-terminal domain, and Porton says this results in less non-specific binding than previous forms when used in immunoassays or on affinity columns. This modification also exposes a lysine-rich beta-turn repetitive region that allows the molecule to be orientated for easier immobilization.

In addition to its line of anti-mouse and anti-rabbit IgG reagents — available in unconjugated, fluorescein-conjugated, horseradish peroxidase-conjugated and biotinylated forms — Vector Laboratories has a set of **multiple-labelling protocols** that explain how to use more than one marker in tissue sections (Reader Service No. 117). The procedures offer step-by-step methods for localizing two antigens with different colour precipitates, using Vector's Vectastain ABC system. The methods do not require the primary antibodies to be made in different species, so multiple staining can be accomplished with two monoclonal antibodies or two rabbit antibodies, for example. The proto-



Varian's double-labelling system gives a strong signal.

cols are free from Vector, who has reserved booth 669.

Protein products

As a part of its University Discount Program to help bring high quality HPLC equipment to college and university laboratories with tight budgets, Waters, a division of Millipore, will be showing its Baseline Gradient System in booths from 713 to 814 (Reader Service No. 118). Waters says the Baseline system is particularly suited for **analysing complex biologicals** such as peptides, metabolites and nucleic acids, and is based on an IBM-compatible NEC APC IV personal computer with mouse-driven software. The \$21,900 (US) system consists of a variable wavelength absorbance detector that covers the range of 190-700 nm, a universal injector that allows injection volumes from 1 µl to 10 ml, and two Waters 501 HPLC pumps. Waters says the positive-flow equalization design of the pumps provides a smooth flow that reduces baseline noise during high sensitivity analyses and minimizes mixing volumes during gradient operation.

J.T. Baker Chemical Company has a line of Bakerbond HPLC columns for **separating peptides and proteins** that it says can perform separations in five minutes (Reader Service No. 119). The Bakerbond Scout columns measure 4.6 mm × 5 cm, and are packed with Bakerbond 300 Å Wide-Pore bonded phases, resulting in a backpressure of 200 to 400 p.s.i. at 1.0 ml min⁻¹. The choice of packings includes PEI for anion exchange, CBX for cation exchange, HI-Propyl for hydrophobic interaction, and C₄ for reversed phase chromatography. J.T. Baker, in booths 854 and 856, has priced its columns at \$195 (US) each, to allow them to serve as an alternative to conventional analytical columns in determining the optimum conditions for protein purification. □

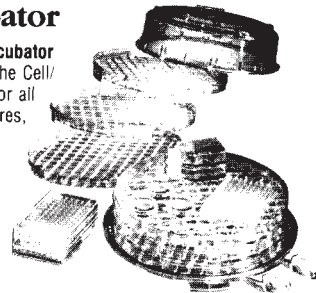
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Reader Service No.28

Graduating into employment

Richard Pearson

The transition from graduation to employment may be longer than the six months allowed for in UK surveys.

GOVERNMENTS the world over are investing in education in the hope that this, along with research and development, will be the key to economic growth. Increasingly, they are also reviewing existing provision with a view to enhancing the vocational element. Education has far broader objectives than simply serving the needs of the economy and its role is crucial in the development and supply of higher-level scientific and technological skills.

There is, however, an inadequate understanding of the nature of the future needs of the labour market on which to base any 'planning', be it by potential students choosing between subjects and courses, institutions planning courses or by governments and agencies wanting to target public finance. One necessary and achievable first step in this direction is an improved understanding of the relationship between education and employment. Valuable input into this comes from the growing body of 'first-destination' and cohort studies of recent graduates; the United Kingdom, Australia, Canada and the United States all regularly monitor this area. But the validity of many of these studies and statistics is debated. In the UK, for example, regular first-destination statistics showing the employment status, or otherwise, of graduates six months after graduation have been collected for over 20 years and are regularly used to show the relative employability of graduates in different subjects¹. These statistics have consistently shown the greater employability of graduates in engineering relative to life sciences and the humanities, and from universities relative to polytechnics. More recently, these figures have been used by the government as a measure of performance for individual institutions. The critics of these indicators argue that, in a rapidly changing market, many graduates are spending a year or more deciding on job and career and hence the six-month picture is becoming increasingly unreliable and misleading.

A recent study of CNAA graduates three years after graduation has shown that, although many more humanities and social science graduates were unemployed six months after graduation, two years later almost all had jobs and the differences between subjects in terms of unemployment was negligible. But technology students earned most, and more arts and humanities graduates considered themselves overqualified. Most important, the study shows that the transition from education to employment in the United

Kingdom can take up to two years, adding further weight to the criticism of the six month figures. What the study fails to show is that subjects are not all equal, in terms of employment prospects. More UK figures should be available at the end of 1987 in the form of the initial results of the Department of Employment's survey of the first five years post graduation of the 1980 cohort of graduates.

What, then, does overseas experience tell us? In Canada, results are now available on the experiences of the cohort of 1982 graduates two years after they grad-

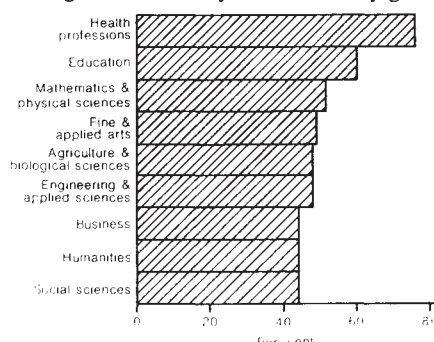


Fig. 1 University graduates rating the match between field of study and job as "very important" by field of study. Source: *National Graduates Survey*, June/July 1984.

uated². This is the second such study (the first was in 1978) and it draws on the experiences of over 35,000 graduates, 73 per cent of whom are either university or college graduates. The short-term picture showed relatively low unemployment rates, at 9 per cent, soon after graduation in January 1983; this figure had fallen to 7 per cent by October of that year but had risen again to 9 per cent by June 1984. In addition some 14 per cent were in temporary work in January 1983, while 19 per cent were not in the labour market and not looking for a job. These figures suggest a more rapid and easier transition into employment than has been apparent in the United Kingdom over comparable years. In Canada, on a subject basis the figures show a remarkable similarity with the United Kingdom, with electrical and mechanical engineers, medics and computer studies graduates all having the highest full-time employment rates at 90 per cent plus, while graduates in humanities, social sciences and biological sciences, along with those in education, had below-average success.

Much is made of the virtues of part-time employment and, although it is rapidly on the increase, it is not very often the first choice of many workers. Among

the Canadian graduates, 7 per cent of men and 13 per cent of women were in part-time employment, most because they could not find full-time jobs; however, for a significant minority it was because they were continuing with their studies. Further study was also the main reason that both men and women were not participating in the labour force. Although unemployment rates averaged only 9 per cent, nearly half the graduates had been unemployed at some time in the first two years after graduation and the average period of unemployment was nearly seven months. This suggests that the Canadians were either more able or more willing to take temporary or stopgap jobs.

If degree subject clearly affects the ease of employability, do graduates want a close link between their subject and their job and to what extent do they achieve it? Such a link was thought to be of highest importance in health and education, and also in maths and physical sciences; fewer, although still almost half, from engineering and business desired a link; while the figure for business was matched by the percentage for the humanities and the social sciences (see Fig. 1). Once in work less than 20 per cent of all graduates in full-time employment said their job was not related to their education and the majority said they needed their qualification. The subjects showing the weakest linkages included the biological sciences, social sciences and the humanities, and it was graduates in these subjects who were also most likely to be in jobs not requiring university qualifications. The strongest linkages were, as expected, in health, business and engineering.

What does this tell us about the linkages between university education and subsequent employment in Canada? First, that most students expect there to be a link but that their rating of the link between subjects does not always follow expectations. Differences between subjects in terms of ease of transition follow a similar pattern to that in the United Kingdom. What is clear from both studies is that the first year or so after graduation is one of transition. However, although statistics based on a six-month snapshot may not reflect the longer-term status quo, the longer-term picture does not show that the differences between subjects change. □

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton BN1 9RF, UK.

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